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I. DEVELOPMENT OF THE RECEPTACULUM OF CLATHRUS COLUMNATUS Bosc.

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(WITH PLATES XI AND XII)

IN working out in *Anthurus borealis* the structural details of its imperfectly known genus, quite unexpected results were obtained with regard to the development of the receptaculum, which made it desirable to include in the investigation other representative genera of the Phalloideæ. A suitable range of additional forms was presented by *Mutinus caninus* (Huds.), *Dictyophora duplicata* (Bosc), and *Clathrus columnatus* Bosc, the two former of these belonging to the subfamily Phalleæ, and the Clathrus and the Anthurus to the Clathreæ. The papers on Anthurus[1] and Mutinus[2] have been published already. With the present paper on Clathrus it becomes possible to present more clearly the important developmental differences between the two subfamilies.

During the winter of 1894–5, a supply of alcoholic material of *Clathrus columnatus*, provided by Dr. Farlow, was studied

[1] A North American Anthurus, its structure and development. *Memoirs Boston Soc. of Nat. Hist.* 3:487. 1894.

[2] The development of Mutinus caninus (Huds.) Fr. *Annals of Botany* 10:343 1896.

under his direction in the Cryptogamic Laboratory of Harvard University. While fully substantiating the conclusion reached in the case of *Anthurus borealis* with regard to the origin of the chamber- and pseudoparenchymatous tissues of the receptaculum from different systems of primary tissues, nevertheless the range of stages was not great enough for a complete account of the development of the receptaculum. During the summer of 1895, a more abundant supply of young stages of *C. columnatus* was collected for me through the kindness of Professor P. H. Rolfs, of the Agricultural College, Lake City, Fla. With this the investigation has since been completed.

The methods of staining, etc., are given in detail in my former papers.

MATURE STAGE, INTRODUCING THE TERMS TO BE EMPLOYED.

The fructification of *Clathrus columnatus* has a receptaculum consisting of from two to five vertically ascending columns, which are quite separate where they arise from the volva, but join together at their apices. Usually there are only four such columns. These are joined together in pairs, the two opposite pairs being then connected together by a short and broad arch of the same nature as the columns. In the earlier stages the receptaculum is compressed into a small space in the interior of the fructification and enclosed by a fleshy bag called the volva. In such early stages the fructifications of this, and other members of the Phalloideæ, are called "eggs" on account of their general appearance.

By the time the spores mature, the egg attains a diameter of from 3 to 5^{cm}. The receptaculum then elongates and bursts out through the apex of the volva, thus raising the spores to a height of from 5 to 8^{cm} above the surface of the ground for more favorable dispersal. After elongation of the receptaculum, the spore-mass, or gleba, as it is called, may be seen as a spherical mass in the upper part of the main central cavity of the receptaculum, hanging from the under side of the arch and the proximate portions of the columns.

Of the three layers of the volva, the middle, or gelatinous layer, is not one continuous sheet as in the Phalleæ, but consists of as many meridionally arranged masses as the receptaculum has columns. These gelatinous masses alternate in position with the columns and are completely separated from each other by the cortical plates (*C'*, *figs. 4*, *5*, and *6*). The cortical plates extend from the base to the apex of the egg and connect each column of the receptaculum throughout its entire length with the outermost, or cortical layer (*C*). The peculiar arrangement of alternating cortical plates and gelatinous masses arises in the early differentiation of the egg, as will be shown further on.

COURSE OF DEVELOPMENT.

The eggs are borne at the ends of short branches of the subterranean mycelial strands. In cases where the egg has arisen as an outgrowth on the side of a mycelial strand, the portion of the strand beyond the egg seems to have ceased its further growth so that the egg becomes practically seated at the end of the strand running into it. The mycelial strands consist of two systems of tissues: a central or medullary bundle of fine hyphæ running in a longitudinal direction, and an outer or cortical layer of coarser hyphæ forming a loose but very interwoven structure.

The cortical layer of the strand is continued upward in the egg as its outer covering (*C*, *fig. 1*). This figure is from a median longitudinal section of an egg 1.5^{mm} long by about 1^{mm} in diameter. The more compact medullary bundle is marked *M*. As in the strand, so here, its hyphæ run in a prevailingly longitudinal direction lying close together. By the double stain used these two layers of the egg are sharply separated from each other in color as well as by the more open structure of the cortical layer. Although so well marked, still they are in intimate connection in their region of contact by means of hyphæ which spread out laterally from the medullary layer and branch and interlace with the cortical hyphæ and become indistinguishable from them. In *fig. 2* a cross-section of an egg in the same stage

of development as that of *fig. 1* is given under the same magnification. The medullary bundle *M* is nearly circular in cross-section.

A developmental change now sets in through which the outline of the medullary portion becomes lobed. These lobes alternate in position with the later formed columns of the receptaculum and extend longitudinally from near the base of the egg almost to the apex of its medullary bundle. The four such lobes usually formed in *C. columnatus* are shown in the cross-section of an egg in this stage (*G*, *G*, *fig. 4*). *Fig. 4* is drawn with the same magnification as *figs. 1* and *2*, the diameter of the egg having become only slightly greater. Many eggs of about the same diameter as these were sectioned and examined in order to find intermediate stages between those of *figs. 1* and *2* and *fig. 4*, which would show the mode of differentiation of the medullary lobes *G*, *G*. It seems probable that their differentiation occupies only a short interval of time, for only one egg in the intermediate stage was found. It is shown in cross-section in *fig. 3*, and under the same magnification used in the other cases. In this stage (*fig. 3*) the medullary and cortical layers are less sharply distinct from each other than in the earlier or later stages. At three points, perhaps four, hyphæ seem to be invading the cortical region of *fig. 2* and forming masses (*G*). These masses are the rudiments of the gelatinous layer of the volva of later stages. Only three such lobes can be made out with certainty in this stage, while four are present in the more advanced stage of *fig. 4*. The absence of the fourth may indicate that the differentiation of all four lobes does not begin at exactly the same time; but it seems more probable, however, that this is an early stage of a Clathrus having three columns for its receptaculum. In such a plant only three gelatinous masses are present in the gelatinous layer of the volva. Specimens having only three columns did occasionally occur in this material.

The rudiments (*G*, *G*, *G*, *fig. 3*) of the gelatinous layer of the volva are most intimately connected with the central medullary mass and must undoubtedly be regarded as belonging to the

medullary system, as they have been heretofore.[3] As shown in this stage the manner of formation of these lobes seems to be that of a general growth of medullary hyphæ along three or four longitudinal lines outward among the cortical hyphæ of the layer *C* of the youngest stage. That this is the actual mode of formation of the lobes is shown by the fact that their marginal portions are less sharply set off from the cortical layer than is the case in later, and even earlier stages. In this stage many cortical hyphæ can be followed into the marginal portions of the lobes, and the color reactions by the double stain used are less sharp in those portions than they are between the cortical and medullary systems in other stages.

It is to be observed that in this stage the surface of the egg does not conform to the surface of the medullary portion, but has in cross-section the nearly circular outline of the youngest stage (*fig. 2*). In later stages a gelatinous accumulation in the lobes (*G*, *G*) causes them to swell outward and laterally towards each other so as to give to the egg a surface with a broad rounded longitudinal ridge, extending outside of each lobe from the base of the egg nearly to its apex. These lobes are separated by shallow furrows, each of which marks the position of a column of the receptaculum, and is of great help in orienting the eggs for sectioning. Were such longitudinal ridges and furrows present on the surface of the egg in the stage of *fig. 2*, they would have favored the view which I formerly held that the medullary lobes originate by outward protrusion of medullary tissue along four longitudinal lines, such protrusion being due to vigorous growth within the medullary portion merely pushing the cortical layer further outward in those regions.

The columns of the receptaculum arise in the angles (*C'*, *C'*) between the medullary lobes (*G*, *figs. 3* and *4*). Ed. Fischer[4] in his study of *Clathrus cancellatus* has called the tissue *C'* of these angles *Zwischengeflecht*, and has referred it to the medullary sys-

[3] *Cf.* Ed. Fischer, Untersuchungen z. vergleich. Entwicklungsgeschichte u Systematik der Phalloideen. Denkschr. d. Schweiz. naturf. Gesellsch. 32: 4. 1890; also Burt on Anthurus, *l. c.* 494.

[4] *Ibid.* 4.

tem of tissues, although pointing out that its hyphæ are coarser, more highly refractive, more loosely arranged, and more irregularly intertwined than is the case in the rest of the medullary system. In all of these points of difference which have been enumerated, the tissue in question agrees with that forming the surface of the egg. As it is also more intimately connected with such cortical tissue than with the medullary tissue, stains the same as the former, and in the youngest stages (*figs. 1* and *2*) is the direct continuation upward in the egg of the cortical tissue of the mycelial strand, it should be regarded as belonging to the cortical system. It will be referred to in this article as the tissue of the cortical plates, as in my earlier paper on Anthurus.

In the earliest stages the cortical layer is closely adnate to the medullary layer. In the stage of *fig. 4* separation of these two layers begins along the inner edge of each cortical plate, the two tissues seeming to be pulled slightly apart, although very numerous hyphal connections still exist between the two surfaces. In the older stage of *fig. 5* this separation has become more complete and a decided fissure has been produced between the two systems along the inner edge of the cortical plate (*C'*). While it is possible that the rapid growth of the medullary lobes (*G*) and their distention with the gelatinous accumulation may have carried the cortical system outward bodily and, to some extent, may have loosened the cortical plates from their connections along their inner edges, it seems more probable that the separation has been caused chiefly by changes in the medullary structure facing against the cortical plates, as shown in *fig. 5*. Medullary hyphæ reaching to the edge of a cortical plate become swollen at the outer end and become arranged side by side in a palisade-layer. Branches of a similar nature crowd their way in between the members of this palisade-layer and so increase its surface that the layer becomes thrown into folds (*t*, *fig. 5*) and torn away from its connections with the edges of the plates (*C'*). In some of the sections of this stage occasional hyphal connections of this kind still persisted.

Examination of the surface of contact of medullary lobes (*G*) of the volva with the cortical layer (*C*) and the cortical plates (*C'*) shows that the hyphæ in this surface now lie in the plane of the surface, indicating that, in the great increase in the volume of these lobes since the stage of *fig. 4*, their distention (partly due to gelatinous accumulation no doubt) has been pushing their surface bodily against the cortical layer. The same distention of these lobes has also brought them closer together, laterally compressing the masses *C'* of *fig. 4* into the narrower plates of *fig. 5*, and into still narrower plates in the more advanced stage of *fig. 6*.

With the further growth of the egg, the medullary surface (*t*, *fig. 5*) becomes thrown into a very complicated series of folds, causing passages to extend in a labyrinthine manner into the main central medullary mass. The cells of the palisade-layer facing the deeper lying passages differentiate into basidia and bear spores. This portion constitutes the gleba (*Gl*) of older stages. Hyphæ from the cortical plates penetrate into the passages situated in front of the edges of the cortical plates, become adnate to the surfaces of the medullary masses (*t*) forming the walls of those passages, differentiate into pseudoparenchyma, and prevent the differentiation of basidia and spores on the surfaces covered. The pseudoparenchyma so formed constitutes the walls of the receptaculum of later stages; the medullary tissue (*t*) enclosed by these walls is the tissue of the chambers of the receptaculum, and it gelatinizes and becomes torn up in the elongation of the receptaculum, leaving the chambers empty for the most part. The relation of these tissues to each other are represented in *fig. 6*. At the right the medullary tissue (*t*) is shown with its hymenial layer of basidia lining the cavities or passages of the gleba. Just to the right of the cortical plate (*C'*) a column of the receptaculum is developing; cortical hyphæ from *C'* have grown into the passages between the medullary masses (*t*) and, in contact with those masses, are developing into pseudoparenchyma. The depth to which the cortical hyphæ have invaded these passages is shown

by the position of the continuous line *q* in the figure. Beyond that line basidia line the passages. At *r*, *r*, *r*, medullary masses may be seen crossed by the line; these masses lie partially in the gleba and partially in the column. In their glebal portion they bear a layer of basidia; on the ends in the column they are covered with the cortical tissue. It is by such connecting medullary masses that the gleba hangs suspended within the cavity of the receptaculum after elongation of the latter.

In the same figure (*fig. 6*) many medullary masses (*t*) may be seen in the column not connected with the other masses to the right. These unconnected masses are in general smaller toward the edge of the cortical plate (*C'*) and, in some stages, they are more closely surrounded by the adhering cortical tissue than are the masses at a greater distance from the edge of the plate. Ed. Fischer has described the occurrence of such isolated masses,[5] which he calls hyphal knots (*Hyphenknäuel*) in *Clathrus cancellatus*, and has concluded that they arise from the differentiation of the tissue of the cortical plates.[6] In this opinion I cannot concur. As already stated, my preparations show that a continuous cavity is first formed between the edges of the cortical plates and the medullary tissue. Hyphæ from the one side of this cavity grow into it. Along the opposite side of the cavity branching masses of medullary tissue extend into the cavity, partially filling it and causing its irregularity in form. Such medullary masses are represented by the dark areas in *figs. 10–13*. They are highly gelatinous, having the same microscopic structure as that of the gelatinous layer of the volva and the main central mass of medullary tissue, and they take the same orange-red stain in my preparations. *Figs. 10–13*, in the order of their numbering, represent serial cross-sections, of which *fig. 10* is of the section at the lower end of the series, or nearest the base of the egg. They are from below the level of the gleba. The examination of such a series of cross-sections affords reason

[5] *Ibid.* 5, *figs. 3* and *4*.

[6] Fischer calls this tissue *Zwischengeflecht*. It is, however, a wholly different tissue from that to which he applies the same name in the Phalleæ.

for believing that these gelatinous masses are all connected with each other and with the main central mass of medullary tissue. In *fig. 10* an irregular mass occurs consisting of five main parts, each of which is marked *1*. Upon following this mass upward through the series, it is found that its five parts finally become separate from each other, and that the outermost part of the original mass does not reach up into the plane of the highest section (*fig. 13*). The small mass marked *2* also fails to reach up into that section. The attempt to follow in serial sections the apparently isolated masses (Fischer's "hyphal knots") of any one section leads to the conclusion that such masses are portions of a highly branched structure arising from the inner main medullary mass, and that the ramification of this mass is outward and chiefly upward. Such a branched structure along the medullary side of the cavity has arisen, without doubt, partly from the folds produced by the formation of the palisade-layer, as already described; but there is evidence that it may be due in part also to an irregular splitting downward and inward into the medullary tissue, as shown by the changes that occur in the connections of the cavity *3* (*figs. 10–13*), and by the fact that masses, joined together into one in some sections, become separate in others, and then join again into one. Other evidence is afforded by the distribution among the medullary masses of hyphæ from the cortical plates. In this stage such cortical tissue is found in greatest abundance in the marginal portions of the cavity; it is almost wholly absent from some, but not all, of the spaces between the more centrally situated masses.

The medullary masses which we have been considering occupy spaces which become the chamber-cavities of the receptaculum in later stages. Although portions of *t*, they are more definitely indicated by *b* in *figs. 6–9*, and *14*.

Walls of the receptaculum.—The tissue of a cortical plate (*C'*) forms a broad layer of loosely interwoven and branching hyphæ, divided into short cells. This tissue is quite similar to that of the cortical layer (*C*) with which it has unbroken connection. Along the inner edges of the cortical plates, their tissue passes

into the cavity which has formed there, and spreads about in it, and between the medullary masses (*b*), and over the surfaces of those masses and on that of the cavity. For some reason (which may, perhaps, be proximity to a supply of available food) these cortical hyphæ find the conditions for their further differentiation most favorable on the surfaces of the masses of gelatinous tissue, and they become closely adherent to such surfaces and grow very luxuriantly there. Still, many of the hyphæ are found in the more open space, running irregularly through such spaces or crossing from one side to the other. It is this tissue which finally fills the spaces between and about the gelatinous masses and becomes the chamber walls of the receptaculum.

An older stage of the rudiment of a column of the receptaculum and of the tissues about it is shown in cross section in *fig.* 7. On the right is the tissue of the gleba with its chambers lined by the hymenial layer (*H*). On the left is the inner edge of a cortical plate (*C'*); its hyphæ may be seen passing among and against the medullary masses (*b*) of the future chambers. These cortical hyphæ are becoming laterally inflated in this region and are plainly recognizable as early stages of pseudoparenchyma. Along the surfaces of the larger cavities, the development of these hyphæ is giving rise to pseudoparenchymatous plates (*p*, *p*) which in later development have the intervening space more filled in with this tissue, forming a more compact partition wall. Near the gleba, large masses (*b*) of the tissue of the chambers are connected, as in *fig.* 6, with the tramal tissue (*t*) and with the tissue on the inner flanks of the column of the receptaculum, tissue just on the border between the gleba and the gelatinous layer of the volva. At places in the cross-section where the two systems of tissues come into most intimate contact and where the plane of the section may happen to give a cross-section of the pseudoparenchymatous hyphæ, it becomes very difficult to determine the true relations to each other of the adnate tissues *p* and *b*. I have examined such places with the utmost care and find no connections between the tissues of the chambers and the pseudoparenchyma of the walls. If such

connections exist in *C. columnatus*, they should show as distinctly as they do in *Mutinus caninus* or in *Dictyophora duplicata*.

At other places in the preparation the pseudoparenchymatous hyphæ may happen to lie in the plane of the section. In such places, in preparations double-stained with paracarmine and safranin, the true relations of the pseudoparenchyma and the tissue of the chambers become clearly shown, as in *fig. 8*. In this figure the wall (*p*) is shown with the tissue of a chamber (*b*) on one side and with the gelatinous layer of the volva (*G*) on the other. The pseudoparenchyma of the wall has no connection with either the tissue of the chamber or with that of the gelatinous layer of the volva. It has merely taken possession of a space between the two. If this figure is compared with *figs. 11*, *12* and *15* of my paper on *M. caninus*, the contrast between the Clathreæ and the Phalleæ in the origin of the pseudoparenchyma becomes very evident. There is also a difference in appearance between the gelatinous tissue in the chambers of *C. columnatus* and that in the chambers of the Phalleæ which I have seen. In the former there is a very marked resemblance to the tissue of the gelatinous layer of the volva. This may be seen also in *fig. 8*.

Ed. Fischer[7] has asserted that the pseudoparenchyma of the walls of Clathrus is homologous with the hymenial layer, being merely a sterile hymenium. The portion of the section drawn in *fig. 9* is of great importance in this connection. It represents a portion of a column and the adjacent gleba. In the upper part of the figure, the basidia of the hymenium (*H*) are seen projecting radially outward from the tramal tissue (*t*) into a chamber of the gleba. The wall (*p*), consisting of pseudoparenchymatous hyphæ, is situated between the tramal tissue and the gelatinous tissue (*b*) of a chamber. The hyphæ of the wall are slightly laterally inflated. They are not connected with the tissue of the chamber or with the gleba. If the homology put forth by Fischer were well-founded, the pseudoparenchyma should have the same connection with the tissue of the chamber

[7] *Ibid.* 7.

that the basidia have with the tramal tissue, and should also project from the chamber surface perpendicularly outward into the cavity between the chamber masses.

Advanced stages.—A median longitudinal section through an old egg is given in *fig. 14*. The section is also median with respect to the columns of the receptaculum, one of which is shown on the right. The beginning of elongation of the receptaculum has torn the glebal mass away from the lower part of the medullary mass. Near the upper end of the column, along its inner side, many more openings show in the wall than further down toward the base. Through these openings the medullary tissue passes into the chambers and firmly attaches the gleba to this portion of the receptaculum. The figure shows how in the mature stage the ball of glebal tissue comes to be suspended from the under side of the apex of the receptaculum.

A more important point presented by *fig. 14* is the manner in which the cortical and medullary systems of tissues are dovetailed together in the receptaculum. From the left an originally continuous mass of medullary tissue sends branches towards the right; from the right an almost continuous mass of pseudoparenchyma sends branches towards the left, filling in all space between the first set of branches.

This section does not show many of the openings in the wall near the base of the receptaculum, through which the medullary hyphæ pass into the lower chambers. Openings of that nature do, however, exist, although they are not so numerous there as higher up. One such passage into a chamber is shown on the left side of the figure.

REFERENCE TO ANTHURUS BOREALIS BURT.

In my account of the structure and development of *Anthurus borealis*, one of the Clathreæ, it was shown that cortical plates extend from the base of the egg upward almost to the apex, and inward from the cortical layer to the receptaculum. In the region below the level of the base of the arms, the inner edges of the plates are united into one cylindrical mass which forms a

sheath of cortical tissue (the cortical sheath) surrounding the stipe for its whole length.

The pseudoparenchyma of the walls of the stipe and of the arms was found connected with and arising from the tissue of the cortical sheath and cortical plates. Medullary tissue from the main central mass was found passing into the chambers of the receptaculum at the base of the stipe and then passing upward from chamber to chamber. At the base of the arms, the ascending masses of medullary tissue are collected into six large masses, each of which is the gelatinous tissue of an arm. No connection could be found between the pseudoparenchyma and the gelatinous tissue of the chambers nor did the pseudoparenchyma stand out from the sides of the chambers so as to indicate any such connection.

The conclusion was reached that in *A. borealis* the receptaculum is formed by the joint action of both cortical and medullary tissues; that the cortical constituent develops into the pseudoparenchyma of the walls, and that the enclosed medullary bundles finally become gelatinous and disappear, thus forming the chambers of the wall.

CONSIDERATION OF FISCHER'S VIEW OF THE ORIGIN OF THE RECEPTACULUM IN THE CLATHREÆ.

Ed. Fischer[8] has stated in his study of the development of *Clathrus cancellatus*, to which references have been made:

1. That the fundament of the receptaculum consists of knots of hyphæ separated from one another by small spaces.

2. That these hyphal knots are formed from the *Zwischengeflecht* (in this case the tissue of the cortical plates *C'* of my figures).

3. That the tips of the hyphæ composing the knots radiate outward from the knots into the narrow spaces between the knots and become constricted and abjointed into pseudoparenchyma, thus forming a plate about each knot.

4. That the tissue in the central portions of the knots

[8] *Ibid.* 5–8.

becomes gelatinous and finally disappears, giving rise to the chamber cavities.

5. That since the tissue of the knots is found to be in connection with the tramal tissue of the gleba at some points, therefore it follows that the receptaculum and the gleba are homologous, that the tissue of the chambers of the former is identical with the tramal tissue of the latter, and that the pseudoparenchymatous walls of the receptaculum chambers are homologous with the hymenial layer of the gleba.

Comparison of Fischer's *figs.* 5 and 7 with my *figs.* 6 and 7 shows that we both see the same general structure and that our difference is in its interpretation. That Fischer's interpretation of the structure is not the correct one seems to me to be shown by the following considerations:

1. The existence in the Clathreæ of the supposed hyphal knots is very doubtful. My experience is that if the attention is fixed upon one of them, and this is then followed through section after section of the series, the supposed "knot" is soon found to be connected with other masses, and so on with the main medullary mass.

2. The tissue of the cortical plates *C'* (*Zwischengeflecht* of Fischer) does not form hyphal knots. About small medullary branches located in the densest portion of the tissue *C'*, as at *2*, *fig.* 9, it often happens that the plane of the section will be unfavorable for displaying the true relations of the two closely adnate tissues. At such a place one needs all of the aid to be had from good double-stained preparations.

3. If pseudoparenchyma of the chamber walls arises from the hyphæ of the chambers by the projection of the swollen hyphal tips outward into the narrow intervening spaces, it ought to be found distinctly connected with the tissue of the chambers, as in the Phalleæ, and its short hyphæ should also be found projecting from the sides of the chamber masses into the narrow spaces in directions perpendicular to the surfaces of the chambers. Such a direction of the pseudoparenchymatous hyphæ should be shown distinctly, as it is in the Phalleæ. In

some parts of the section, where the pseudoparenchymatous hyphæ are closely crowded against the chamber tissue, and where the plane of the section has cut these hyphæ transversely, one must be on his guard against mistaking the cut ends of adjacent pseudoparenchymatous hyphæ for pseudoparenchymatous bodies standing out perpendicularly from the sides of the chambers. Such closely crowded cut ends, with their looser arrangement toward the middle of the space, form a block-like structure very well adapted for giving the familiar optical illusion of radiation.

I fail to find hyphal connection between the pseudoparenchyma and the tissue of the chambers, nor is there an arrangement of the pseudoparenchymatous hyphæ perpendicular to the surface of the chambers. On the contrary, wherever the plane of the section discloses the arrangement, these hyphæ are found lying parallel with the surfaces of the chamber masses, as shown in *figs. 8* and *9*. If Fischer's theory were true, such an arrangement should not be found at any point.

4. Since the homology claimed between the pseudoparenchyma and the hymenial layer of Clathrus depends upon the origin of pseudoparenchyma from the tissue of the chambers, it ceases to be tenable.

The theory of the origin of the receptaculum enunciated by Ed. Fischer has been accepted by A. Möller[9] in his interesting and instructive work on the Brazilian Phalloideæ. His investigation seems to have been completed and his work in process of publication when my paper on Anthurus reached him, as his references to it are in footnotes.

SUMMARY FOR THE CLATHREÆ.

In the earliest stage, the egg consists of cortical and medullary systems continued upward from the mycelial strand.

The cortical layer gives rise to the outer layer of the volva, the cortical plates, and the pseudoparenchyma of the receptaculum. The medullary portion gives rise to the gelatinous masses

[9] Brasilische Pilzblumen. Jena, 1895.

of the gelatinous layer of the volva, to the gleba, and to the gelatinous tissue of the chambers of the receptaculum.

In such differentiation, the gelatinous masses of the volva are the first to be set off. They arise by growth outward into the cortical region of the hyphæ from several areas of the medullary mass.

The masses of cortical tissue which separate the medullary gelatinous outgrowths from each other retain their connection with the outermost layer through all the later development of the egg. They may be called cortical plates; their position is indicated by shallow furrows on the surface of the egg.

Along the inner edge of each cortical plate, a cavity forms by the separation of the two systems of tissue in that region. The medullary tissue facing these cavities gives rise to the gleba and to branching hyphal masses, which project outward and upward into the cavities and become the chamber tissue of the receptaculum.

The receptaculum is formed through the joint participation of the two systems of tissue and differs in its origin and mode of formation from that of the Phalleæ. Its chambers have the position originally occupied by a branched structure of medullary origin, while its pseudoparenchymatous walls form by ingrowth of cortical tissue about and between the chamber masses.

The pseudoparenchyma of the walls is not homologous with the hymenial layer.

The elongation of the receptaculum in *C. columnatus* begins at the base. After elongation of the receptaculum, the gleba hangs suspended from the arch of the receptaculum by medullary tissue constituting chamber masses of the receptaculum.

RELATIONSHIP OF PHALLEÆ AND CLATHREÆ.

The Phalleæ and the Clathreæ, the two usually accepted suborders of the Phalloideæ, resemble each other:

1. In gross structure. Both have a volva of the same general structure, which becomes ruptured by the same means and from which there issues the receptaculum.

2. In microscopic structure. Both have a gleba of the same chambered structure, with basidia lining the chambers and bearing numerous (4–8) very minute spores. Both have a receptaculum of chambered structure, with walls of irregularly laterally inflated hyphæ, the pseudoparenchyma.

3. In biological adaptation for combining a safe early development with a good means of dissemination of the mature spores. In both the early development is subterranean, in the form of a compact egg covered by a thick gelatinous coat retentive of moisture and probably protective. When the spores are mature they are quickly raised above ground, and an attractive lure for insect agency in their dissemination is then offered by the fetid odor and saccharine composition of the deliquescing mass in which they lie, as well as by the bright colors of the receptaculum which supports them.[10]

The differences between these two subfamilies are no less significant. They are shown:

1. In the mature stage. The position of the gleba is outside the receptaculum in the Phalleæ, while it is within that structure in the Clathreæ. The gelatinous layer of the volva is a continuous sheet in the Phalleæ, while in the Clathreæ it is separated by the cortical plates into several more or less connected masses, wholly separate from each other in Clathrus, but joining above into one in Anthurus.

2. In early development. (*a*) In early differentiation of the Phalleæ, the steps by which the lower and lateral portions of the sheaf-like head (those portions bearing the future hymenium) lose their original direct connection with the medullary bundle *M* and become split away, as it were, from the stipe from below, have no parallel in the Clathreæ, and remind one rather of the changes that occur in the formation of the pileus in some Agaricineæ. (*b*) The hymenium of the Phalleæ arises underneath the gelatinous layers of the volva on the inner (under)

[10] (*a*) W. R. Gerard, Bull. Torr. Bot. Club **7**: 30. 1880; (*b*) Rathay und Haas, Ueber *Phallus impudicus*, (L.) und einige Coprinus-arten, Sitzungsb. d. Mathem.-Natur wiss. Akad. zu Wien **87**[1]: 18. 1883; (*c*) Fulton, Dispersion of spores of fungi by insects, Annals of Botany **3**: 207. 1889.

side of the peripheral portions of the sheaflike head, while in the Clathreæ its portions alternate with the gelatinous lobes of the volva. (*c*) The basidia of the young hymenium of the Phalleæ face towards the axis of the plant; in the Clathreæ they face in the opposite direction, towards the periphery. (*d*) In the Phalleæ, tissue of medullary origin gives rise both to the pseudoparenchyma of the receptaculum and to the tissue of its chambers; in the Clathreæ, cortical tissue continuous with that of the mycelial strand forms the pseudoparenchyma,[11] while the tissue of the chambers is of medullary origin and connection.

The marked resemblances that have been pointed out between the mature stages of the Clathreæ and Phalleæ have been regarded in systematic botany as sufficient for joining them into a well-marked order, the Phalloideæ. The difference in the position of the gleba, whether on the outside of the receptaculum or within it, has been made the basis of their separation as suborders.

Rehsteiner's work[12] on *Hysterangium clathroides* and *Hymenogaster decorus*, both belonging to the Hymenogastreæ, led him, on the ground of the position of the hymenium and the direction in which it faces, to point out a probable origin of the Clathreæ from a Hysterangium-like ancestor, and a possible origin of the Phalleæ from one of the Hymenogastreæ near Hymenogaster, a view Ed. Fischer has also since expressed.[13]

Thaxter's account of *Phallogaster saccatus*[14] indicates that this may be a species somewhat closer to the Clathreæ than *Hysterangium clathroides* is, on account of having a gelatinous layer

[11] That the pseudoparenchyma of the receptaculum of the Clathreæ is cortical in origin is not strange. A. Möller states that in *Clathrus columnatus* and *Blumenavia rhacodes* the cortical layer of the mycelial strands is pseudoparenchymatous (Brasilische Pilzblumen 143), and that in the latter species it is so highly pseudoparenchymatous as almost to lose its hyphal characters (*ibid.*, 61). Both of these species are Clathreæ.

[12] Rehsteiner, Beiträge z. Entwicklungsgeschichte d. Fruchtkörper einiger Gastromyceten. Botanische Zeitung. **50**: —(38–40). 1892.

[13] Neue Untersuchungen d. Phalloideen. Denkschr. d. schweiz. naturf. Gesellsch. **33**[2]: 44. 1893.

[14] Note on *Phallogaster saccatus*. BOT. GAZ. **18**: 117. *pl. 9*. 1893.

between its gleba and peridium that is to be regarded, probably, as the forerunner of the gelatinous masses in the volva of the Clathreæ.

A. Möller's description of his *Protrubera Maracuja* makes a still closer connection.[15] He finds not only gelatinous masses next to the peridium, but also finds them arranged alternately with reference to the glebal masses, as in the Clathreæ, and separated from each other by cortical plates along whose inner edges the glebal masses develop. The development of the cortical plates from the peridium, and their connection with it, are made very probable by his *figs. 2–5, pl. VI.*

My studies, showing that the pseudoparenchyma of the receptaculum of the Clathreæ is of cortical (peridial) origin, supply another link in the chain connecting the Clathreæ with Hysterangium. By making known the early differentiation of the Phalleæ, and by showing that this subfamily differs wholly from the Clathreæ in the development of the receptaculum, they make it certain that the Phalleæ, although as highly a differentiated subfamily, cannot have arisen from the Clathreæ.

It seems safe to conclude:

1. That the Phalleæ are not directly related to the Clathreæ.

2. That both subfamilies have arisen from lower forms outside their family.

3. That the Phalloideæ consist of two parallel series of forms, which, through variations from unlike starting points, have attained to highly specialized structures adapted to the same ends.

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EXPLANATION OF PLATES XI AND XII.

All figures were drawn with the aid of an Abbé camera lucida. The following letters are common to all the figures: *C*, cortical layer; *C'*, cortical plate; *G*, gelatinous layer of volva; *Gl*, gleba; *H*, hymenium; *M*, medullary bundle of young stages; *b*, tissue of the chambers; *i*, inner layer of the volva; *p*, pseudoparenchymatous wall of the chambers; *t*, tramal tissue.

[15] *Ibid.* 10.

FIG. 1. Median longitudinal section of the youngest egg. × 60.

FIG. 2. Cross-section of an egg in the same stage of development as that of *fig. 1*. × 60.

FIG. 3. Cross-section of a slightly older egg, in which differentiation of the gelatinous masses of the volva has just begun. It is doubtful whether this would give rise to three such masses or to four, in the former case with a receptaculum having three columns alternating with the masses, or in the latter to one with four. × 60.

FIG. 4. Cross-section of an egg in a still older stage, with four masses (*G*) forming the gelatinous layer of the volva. The four columns of the receptaculum arise in the angles between these masses along the inner edges of the cortical plate (*C'*). × 60

FIG. 5. Portion of a still older egg in cross-section, showing an early stage in the formation of a cavity between the cortical plate (*C'*) and the deep-lying medullary portion (*t*). One column of the receptaculum arises in a part of each such cavity; the hymenium arises along the surfaces of the remaining part. × 60.

FIG. 6. The same region of the egg shown in *fig. 5*, but in a more advanced stage. The column is now developing. The sharp continuous line, *q*, has been drawn to more clearly set off the column from the gleba. At the places marked *r*, *r*, *r*, medullary masses connect the tissue of the chambers of the receptaculum with the tramal tissue of the gleba. × 34.

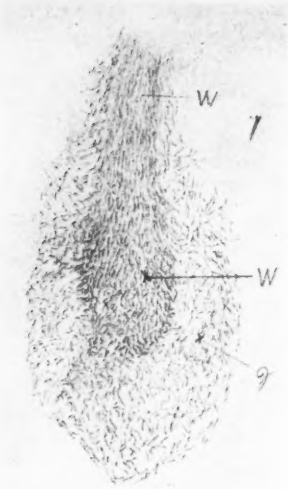
FIG. 7. Cross-section of a fundament of a column of the receptaculum, showing medullary connections of the chamber tissue towards the right and cortical connections of the pseudoparenchyma towards the left. × 34.

FIG. 8. Portion of the wall, *p*, of *fig. 7*, more highly magnified. × 670.

FIG. 9. Portion of the wall, *p*, between the gleba (*Gl*) and a chamber mass (*b*) of *fig. 7*. × 325.

FIGS. 10–13. Serial sections of chamber-masses of a column, with adjacent tissues, in the lower part of the egg. *Fig. 10* represents the lowest section. The series shows how the small and apparently isolated masses ("hyphal knots") are in reality connected with the main medullary mass. × 106.

FIG. 14. Median longitudinal section through an egg in which elongation of the receptaculum is beginning. Shows a column in longitudinal section displaying its relations to other structures. × 6.



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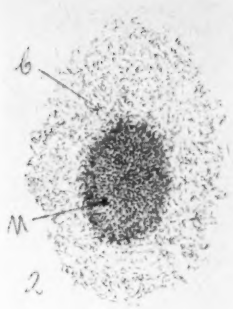

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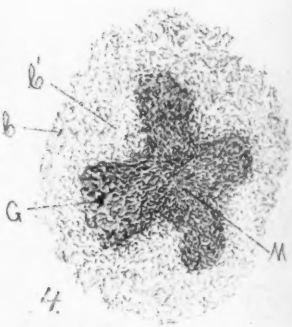

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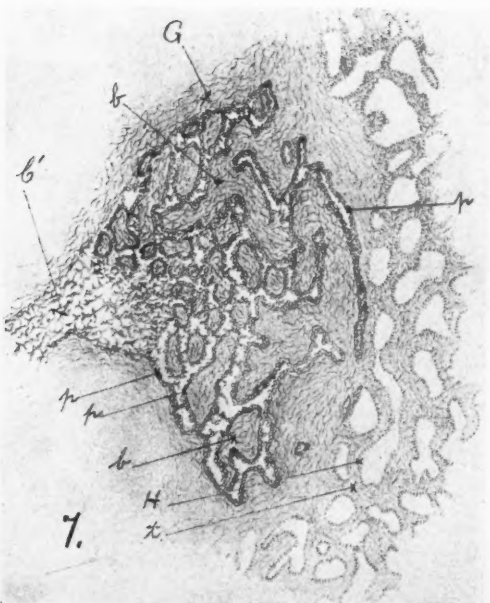

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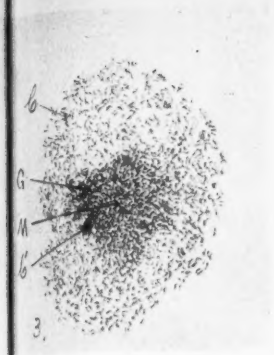
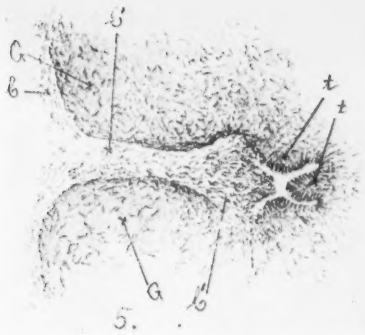

b
G
M
b
3.


b'
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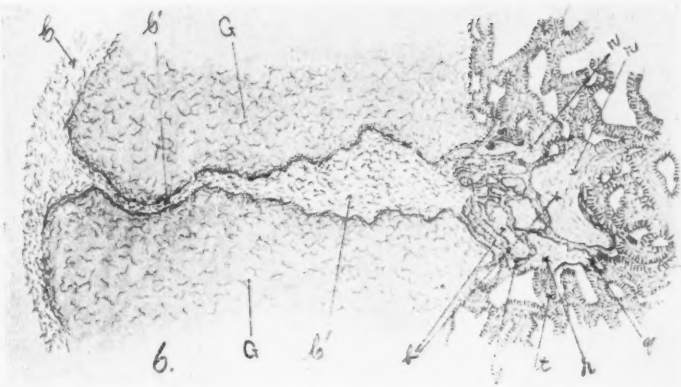
b
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G
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t
h
q

H
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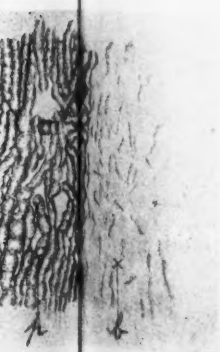
h


h
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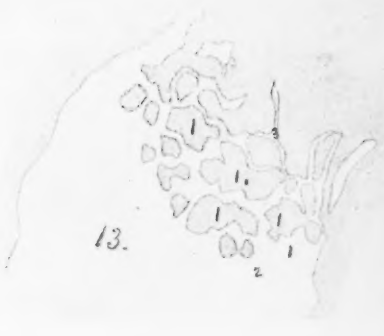
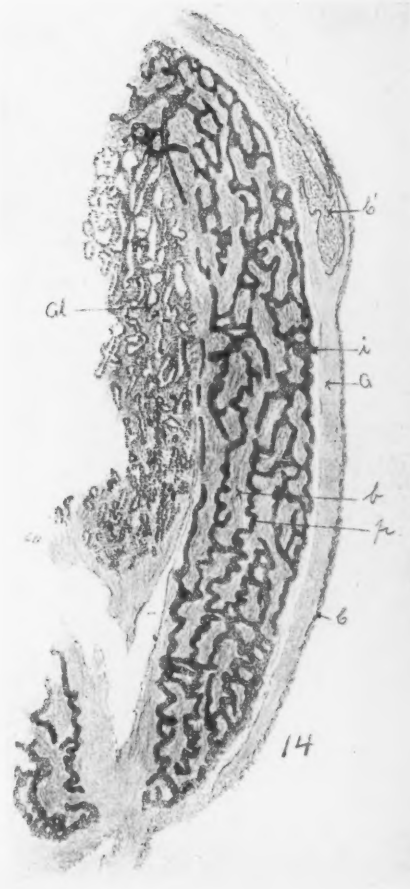




BURT on CLATHRUS.

THE MECHANISM OF MOVEMENT AND TRANSMISSION OF IMPULSES IN MIMOSA AND OTHER "SENSITIVE" PLANTS: A REVIEW WITH SOME ADDITIONAL EXPERIMENTS.

D. T. MacDougal.

(WITH PLATE XIII)

The history of investigation of the transmission of impulses in "sensitive" plants begins with the work of Lindsay on Mimosa in Jamaica in 1790, which for some reason was not published until 1827.[1] During the century following the subject received no attention except that given it in the north temperate zone, in warm houses, and for the greater part under highly artificial conditions. In such manner it has been the object of numerous series of experiments, and of many highly ingenious speculations.

Briefly summarized, the aggregate results of both methods embrace nothing beyond a delineation of the anatomical details, the chemical properties of some of the tissue systems, an immense number of the features of reaction under artificial conditions, "working theories" of the mechanism of the motor organs and metaphysical explanations of the transmission of impulses, and general relation of such highly specialized forms of sensitiveness to the developmental history of the plant.

The work upon the subject under conditions necessarily artificial has been so futile in real results that it has come to be admitted on all hands that a satisfactory solution of the problems presented may be accomplished only by researches prosecuted in the tropics, in the habitat of plants which have acquired a high degree of sensitiveness.

[1] Quart. Jour. Sci. Lit. and Art **24**: 79. 1827, and **25**: 434. 1828.

As an account of such an attempt the recent work of Cunningham deserves attention.[2]

As a basis for the theory upon which this author seeks an explanation of the mechanism of pulvini he devotes a large proportion of his work to the demonstration of the novel idea "that the great majority, if not all, of the transient spontaneous movements of higher vegetable organisms, whether of a nyctitropic character or arising in connection with other conditions than the incidence or removal of sunlight, are not dependent upon the presence of any specially irritable and contractile protoplasts within the motor organs, but on purely physical processes connected either with fluctuations in the osmotic capacities of the tissue-elements, or with alterations in the relations existing between local and general supply and loss of water." In support of this remarkable statement, but very little evidence obtained by an examination of the cell is advanced, but dependence is placed upon the external consistency and color of organs subjected to various reagents. Thus flowers of Hibiscus with the peduncle in water were exposed to ammonia gas in a moist chamber, and as they did not lose their form, while flowers similarly exposed to chloroform wilted, the conclusion was drawn that the osmotic activity of the dead cells was increased by the ammonia in the first instance. A final conclusion derived from similar experiments was that "there is no direct relation between turgidity and the presence of living protoplasm in the turgid elements," but in some instances turgidity may be indirectly dependent on the protoplasm because of the necessity for the manufacture of osmotically active substances; a conclusion certainly at variance with almost all of the known facts concerning the physiology of the cell. Not only does the author deny the possibility of changes in the filtrative properties of protoplasm, but he disregards the simple physical properties of this colloidal substance. He proposes a design

[2] CUNNINGHAM, D. D.: The causes in the fluctuations in the motor organs of leaves. Annals of the Bot. Gard., Calcutta **6**: 1–145. 1895. 4to. 7 lith. col. plates.

for the construction of a machine which shall react similarly to Mimosa.

All pulvinar movements are supposed to be due to a loss of water from the portion of the pulvinus in which contraction ensues, either directly by transpiration, or by withdrawal by the action of neighboring tissue so affected.

Scant attention is paid to the results of Pfeffer and his students, but the author gives the records of a very large number of experiments which, as he rightly points out, show reactions quite different from those obtained in northern latitudes. The thermometric records are meager in certain series, but it is possible that the temperature variations do not exert such an important influence in the tropical habitat of the plant.

According to Haberlandt[3] and others, the transmission of impulses and reaction in Mimosa bears a direct relation to the conditions securing an excessive root supply of water and hindered transpiration. Cunningham, however, finds the relation an inverse one, that plants in a saturated atmosphere react least readily. In a comparison of the three most important forms of stimuli he says: "In cases of 'contact stimulation' we induce mere local distributions of liquid within the tissues; in cases of incision we give rise to temporary exudations from the general supply of liquid; in cases of heating we secure not only this, but in addition we establish temporary increase of transpiratory loss and a site of persistent abnormal drain." In what manner a contact stimulus may cause alterations in the transpiration of a cell without the interposition of protoplasmic action is not explained. It must be said, however, that Dr. Cunningham's observational results are of great value, and are very suggestive as to methods useful in a continuance of the work.

ADDITIONAL EXPERIMENTS.

During the summer of 1895 I was enabled to make a number of experiments with a view of determining the chief factors

[3] Reizleitende Gewebesystem der Sinnpflanze. 1890.

in the transmission of impulses by Mimosa and other plants, in the Botanical Institute at Leipsic by the courtesy of Geh. Professor Pfeffer, to whom I am also indebted for his untiring attention and advice.

A number of the tests were repeated in the Botanic Institute at Tübingen in the present year, and I am indebted to the director, Professor Vöchting, for the opportunity. Some of the tests have also been repeated in the plant houses of the University of Minnesota.

Haberlandt's conclusions as to the transmission of impulses by means of hydrostatic disturbances in a series of elongated cells (the "Schlauchzelle") lying externally to the xylem have been received with general favor, and accounts for such a large number of the phenomena of transmission that certain of the experiments were arranged in such manner as to test the capacity of this theory for final explanation.

In the first place, repetitions were made of the well known experiments in which impulses were transmitted through portions of stems and petioles which had been killed by steam or dry heat in such manner as to allow the dead portions to remain mechanically intact. In my own work this was accomplished by winding soft cloth around the portion to be killed and saturation with water at 90–100° C. for five minutes. In some instances the dead portions were allowed to desiccate and in others a wrapping of tinfoil or a sheath of oiled plaster of Paris prevented undue loss of moisture.

I was able to transmit impulses from an incision or flame through dead portions of stems 3^{cm} in length; in some instances in which desiccation had proceeded to such an extent that the cell lumina of the dead portion were quite devoid of liquid contents, and in one instance through a portion bent at right angles by the weight of the leaf.

I was able to obtain similar transmissions in the midrib of the multipinnate leaf of *Oxalis sensitiva*, which offers many of the features of Mimosa.

In a few instances a reaction was obtained when incisions

were made in the dead portion of a stem or petiole of Mimosa, but no great reliance is placed in such results.

Dr. Cunningham has repeated the tests of transmission through dead portions of stems, and was able to send an impulse through stems consisting of alternating living and dead sections.

The above experiments, giving similar results in the hands of a great number of workers, demonstrate conclusively that transmission may be accomplished in portions of the plant in which no turgid cells occur, and consequently in which no hydrostatic disturbance is possible.

With such facts in evidence the next step was naturally the determination of the question as to whether or not a hydrostatic disturbance constituted an impulse. Three methods were used. A number of young, healthy plants 40^{cm} in height were brought into the experiment room, and after being cut off near the surface of the soil, the bases of the excised stems were set in beakers of distilled water. The immersed portion (10^{cm} long) of the stem was split and stripped in such manner that a large surface composed of active cells was exposed. When the leaves had regained their normal position half an hour later, with the air temperature at 28–30° C., the water in the beaker was withdrawn and quickly replaced by a saturated solution of potassium nitrate without mechanical disturbance to the shoot. Although the endosmotic action of the potassium must have resulted in the almost instantaneous withdrawal of a large quantity of water from the "Schlauchzelle" and other exposed tissues of the base of the stem, no reaction followed. The leaves were found to be in their usual sensitive condition after the experiment, when contact or incision stimuli were applied.

The bases of the stems of small plants were sealed securely into a glass tube 10^{cm} long, 1^{cm} internal diameter, filled with water, which connected at the other end with a leaden tube 30^{cm} long leading into a receiver of a capacity of 4 liters, with a vacuum of 70^{cm} of mercury. When the leaves had regained their normal position, with the temperature at 28–32° C., the stopcock leading into the vacuum was turned, allowing the full

force of the vacuum to act on the base of the stem. In no instance was a reaction obtained. The usual tests showed a normal degree of sensitiveness in the plant.

The two experiments above described must lead one to conclude that diminished hydrostatic pressure does not constitute an impulse. It is, of course, open to belief that greater variations in pressure or a more sudden application of the same might be followed by a reaction.

To avoid a misconception of the effects of such diminution of pressure on the cell contents, attention is called to the fact that the "Schlauchzelle" form a series of continuous tubes, the contents of which freely communicate by openings in the cell walls, and that variations in pressure on any part of the system are quickly distributed through the entire system, as is demonstrated by the following experiment.

In order to test the effect of increased pressure on the plant, shoots were securely sealed into a short section of glass tubing, as above, by means of a rubber stopper bound in place by wires. The tube was filled with water and the other end connected in a similar manner with a leaden tube, with an internal diameter of $3–4^{mm}$ and 1.5^{m} in length, leading to the chamber of a compression air-pump (see plate). When the leaves had regained a normal position in an air temperature of 26–32° C., by a sudden stroke of the handle of the pump a pressure of 3–8 atmospheres was suddenly exerted on the base of the stem, but no reaction followed. That the increased pressure was exerted throughout the plant was proven by the manner in which water poured from the clipped end of a distant leaflet, and that it passed through the "Schlauchzelle" was shown by stripping away the tissues external to these cells. The pressure was thus communicated to distant parts of the plant within a second from the time of its application.

As another test, with its object similar to the above, a compressive pressure was quickly applied to various parts of the plant by the fingers, forceps, or other appliances. In some instances reactions were obtained. It is extremely difficult to

compress the stem quickly without communicating a mechanical disturbance to the plant. It is also difficult to give the compression without crushing the tissues of the plant. It seemed, however, that in the instances where the above faults were avoided no reaction followed the compression, though no conclusions are based on this result.

In conclusion it may be said that the following points are somewhat firmly established: (1) Impulses may be transmitted by Mimosa and Oxalis through dead portions of stems and petioles in which the conditions are such that a transmission by the cell-wall or the water in the wall only is possible. (2) Great variations in the pressure exerted on portions of the plant in such manner as to set up hydrostatic disturbances extending throughout the entire plant are not followed by reactions; hydrostatic disturbance therefore does not constitute an impulse.

It is to be noted, however, that while it is proven that an impulse may be transmitted by a wall of a dead cell, it does not follow that the entire transmission from the point of reception to the motor organ is accomplished by such means alone. It seems quite possible that protoplasmic action plays a part at both ends of the chain connecting the two points, and that while a hydrostatic disturbance does not constitute an impulse, it may play a minor part in its transmission.

The entire problem, together with that of the developmental history of such highly specialized forms of "sensitiveness" as those exhibited by Mimosa, must be followed to their solution in the tropical habitats of the plants.

THE STATE UNIVERSITY OF MINNESOTA.

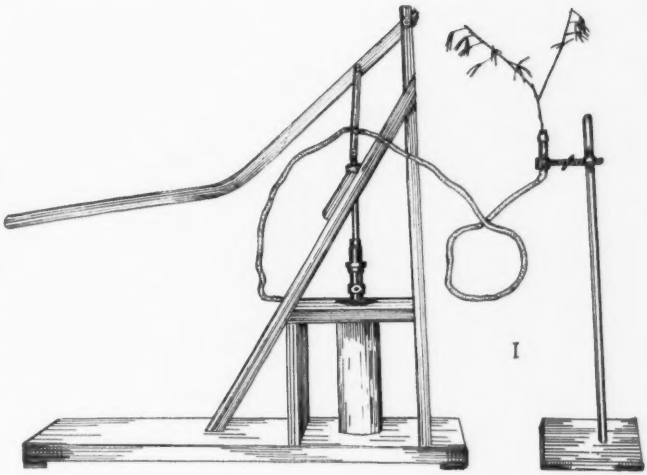
EXPLANATION OF PLATE XIII.

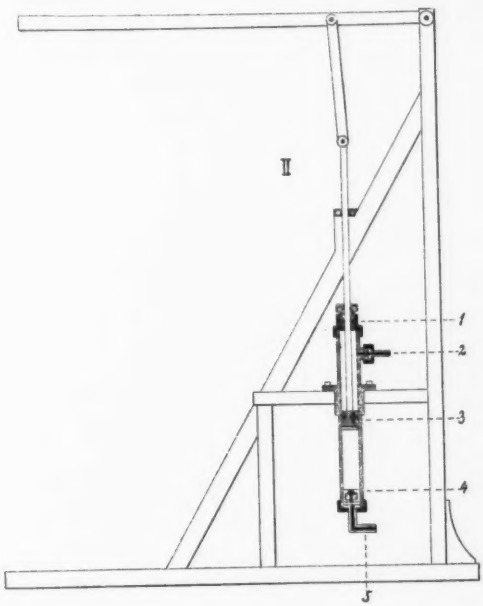
FIG. 1. Showing branch of Mimosa attached to compression pump. A downward stroke of the piston has been given, resulting in the sudden increase of pressure on the base of the excised branch with no reaction of the leaves in consequence. The leaves were then stimulated by a stroke from a pencil, resulting in a normal reaction. The leaden tube in which the

plant is fixed connects with the cylinder at *5* in *fig. 2*. Drawn from a photograph. One-sixteenth natural size.

FIG. 2. Section of compression pump. 1. Fitting around piston rod. The piston rod is driven by the lever handle to which it is connected above. 2. Stopcock leading into the open air; not closed when the pump is used for compression. 3. Piston head moving "air tight" in a metal cylinder. 4. Valve. 5. Outflow pipe through which compressed air is forced. This pipe is extended horizontally in the opposite direction and upward to a point near the base of the crooked lead tube leading to the plant, in the apparatus shown in *fig. 1*. At a point near the juncture with the lead tube it is furnished with a stopcock leading into the open air, which is to be closed during the compression stroke and opened when the stroke is reversed. In *fig. 1* the cylinder is surrounded by a safety casing not shown in *fig. 2*. One-eighth natural size.

The plate for *fig. 2* was copied from the original drawing of the apparatus, and was kindly furnished me by the designer, Mr. Eugene Albrecht, Universitäts-mechaniker, Tübingen, and I am indebted to him for permission to reproduce it here.





MACDOUGAL on a COMPRESSION PUMP.

THE MORPHOLOGY AND DEVELOPMENT OF CERTAIN PYRENOMYCETOUS FUNGI.

MARY A. NICHOLS.

(WITH PLATES XIV-XVI)

THE study of the morphology and development of the ascomycetous fungi comprises four essential questions: (1) sexuality; (2) structure of sporocarp; (3) origin and development of spore; (4) presence, structure, and behavior of nuclei. The literature includes the results of many valuable investigations dealing with one or more of these questions throughout the great class Fungi. The thoroughness and accuracy of this work has advanced steadily with the improvement of methods, and much of the earlier work has been disproved later. In the meantime two opposed schools have arisen, one maintaining the sexuality of the higher fungi and their relation to the Florideæ, the other denying the presence of sexual organs and tracing the development of the compound sporocarp through an asexual line of ancestry.

Throughout the Phycomycetes there remains little doubt of the existence of a distinct sexual process. Representative forms in the different families have been described by De Bary (1) and many others. The structure of the sporocarp, and origin and development of the spore in this group are comparatively simple processes, and have been more or less thoroughly demonstrated. Nuclear phenomena here have also received some attention. Among the later and more complete articles may be cited that of Istvánffy (2) treating of numerous different species.

The Mesomycetes (Brefeld) seem to lend themselves to either line of development. Those leading toward the Ascomycetes furnish some instances of undoubted cytoplasmic fusion at least, while in the Ustilagineæ and Uredineæ Dangeard (3) and

Sappin-Trouffy (4) describe certain nuclear fusions which they explain as sexual processes. No details of nuclear structure and division in these forms have as yet been described.

In the Basidiomycetes the only process analogous to conjugation is the fusion of nuclei in the basidium before the formation of spores. Nuclear studies in this group have been more numerous and complete than elsewhere among the fungi. De Bary (1) in three species has observed the presence of nuclei in the basidium. Rosenvinge (6) has demonstrated the same in thirty-five species. Strasburger (7) has observed the nuclei in Agaricus and found that they divide. Wager (8) in 1893 published the results of extensive nuclear studies upon Agaricus and Amanita. According to his statements, the nuclei of the basidia fuse in pairs before spore formation, and after this fusion successive bipartitions of this fused nucleus occur to furnish nuclei for each of the four sterigmata. During division the nuclear membrane is gradually dissolved and the nucleolus and chromatin masses left free in the cytoplasm. The latter have previously arranged themselves in an equatorial plate. A spindle is now formed, at the poles of which are dark rounded bodies, probably centrosomes, but he fails to find any radiating striæ. During the division the nucleolus disappears. After division of the chromosomes the spindle disappears and the daughter chromosomes at either pole fuse together and, he states, "apparently form the daughter nucleole." At the same time the linin network becomes more strongly differentiated, new membranes are formed, the daughter nuclei assume the size and appearance of the parent nucleus and are again ready to divide.

In the Ascomycetes minute study becomes much more difficult, the investigations are more meager and less reliable, and the conclusions are more at variance. Of the generalizations made by De Bary (1) and others it is only necessary to call special attention here to those on the sphæriaceous Pyrenomycetes. De Bary cites only Xylaria and Sordaria as having unmistakable sexual organs, and in these conjugation has not been observed. In Xylaria the archicarp, he says, seems to disappear before the

formation of the asci, and hence no relation can be traced between fertilization and the origin of the spore. In Sordaria also, according to Woronin (9), the origin of the ascus cannot be traced certainly to the archicarp. "In Claviceps, Epichloe, Pleospora, and perhaps also Nectria, no cooperation of the above named organs (archicarp and antheridium) has been observed, and no distinct ascogonium." . . . "The young perithecium, as at present known, is a body consisting of similar hyphæ or parenchymatous cells, and its elements are gradually fashioned and differentiated into the parts of the perithecium."

"Hartig's (10) conjecture with regard to Nectria, that special ascogenous initial organs are really present on the very young stoma, but up to the present time have been overlooked, may certainly hold good of Claviceps and Epichloe." (De Bary, *l. c.* 200.) Hartig (10) finds sexual organs in Rosellinia. Woronin (9) claims sexuality for Sphæria and Sordaria, and Harper (5), in a recent article on Sphærotheca, shows conjugation and traces the origin and development of the ascus from the fertilized archicarp.

Thus far we have shown strong support for the theory of the presence of sexuality in the Pyrenomycetes. But Brefeld (11), with a school of well known workers, opposes this view, tracing the development of the compound sporocarp through an asexual line and denying the significance which the De Bary school attach to certain characteristics of these plants.

Concerning the nuclei of the Ascomycetes we learn from De Bary that the young asci in both Discomycetes and Pyrenomycetes, so far as studied, contain each a primary nucleus, and later one smaller nucleus is present in each of the eight spores. Sadebeck (12), in 1883, published certain details of nuclear behavior in the Exoascaceæ. He indicates that karyokinetic divisions take place in the ascus, increasing the number of nuclei from one (the primary nucleus) to one for each of the spores. His karyokinetic structures are, however, very rudimentary, and are represented in his figure by two rounded granular bodies at the poles of a spindle consisting merely of three lines. No details of karyokinesis are given.

Fischer in 1885 goes somewhat more deeply into detail regarding the structure of the nucleus in Exoascus. According to his observations the nucleus is a round granular mass in which smaller and darker rounded bodies appear just before division. Next a spindle is formed, consisting of four threads converging slightly toward the poles. In the equatorial plane of the spindle four chromosomes now appear, which divide and pass to the poles of the spindle, where they form themselves into daughter nuclei. This description is of course crude, as were the methods of observation upon which it was based. Gjurasin (13) in 1893 published the first detailed account of karyokinesis in the Ascomycetes. It is based upon a study of *Peziza vesiculosa*. He describes the nucleus as consisting of a distinct round granular nucleolus surrounded by a layer of hyaloplasm in which an indistinct network is present. At the time of division the nucleus elongates, the nucleolus becomes eccentric, the chromatin aggregates into small masses, and two centrosomes with radiating striæ appear. Between these are drawn the nearly parallel threads of the spindle. The chromatin masses collect in the equatorial plane of the spindle and divide, and the daughter chromosomes very quickly seek the poles and soon are surrounded by new nuclear membranes, the membrane of the parent nucleus having meantime disappeared. In these daughter nuclei the nucleoli appear after the formation of the membrane, and with their appearance the mother nucleus, which up to this time has persisted lying free in the cytoplasm, disappears. Second and third divisions occur in like manner. Gjurasin states that he spent two years in the search for these karyokinetic structures, obtaining his final results by means of the Hermann and Flemming methods of fixing and staining.

Harper (5) in 1895, by methods similar to those adopted by Gjurasin, observed karyokinesis in *Peziza Stevensoniana* Ellis, and *Ascobolus furfuraceus* Pers. From studies of these and various allied Discomycetes he concludes that the members of this group are especially favorable for the study of nuclei in the ascus. The results of his work may be summarized as follows:

there exists in the young ascus four nuclei which fuse in pairs to form a single primary nucleus. This nucleus divides in a true karyokinetic manner to furnish a nucleus for each spore. In the process of division (1) the chromatin collects in masses on the network; (2) the nucleolus becomes eccentric; (3) centrosomes with radiating striæ appear; (4) the chromosomes arrange themselves in the equatorial plane, divide in halves and seek the poles; (5) the nuclear membrane is ruptured and the threads from pole to pole become very much elongated and parallel. The nucleolus meantime has gradually diminished and by the time the daughter nucleoli are formed it entirely disappears. The daughter nuclei attain normal size and structure and undergo two further successive divisions, providing one nucleus for each spore.

The foregoing summarizes briefly the work so far done along the lines of morphology and development in the higher fungi. As may be seen, the first two divisions of the subject (sexuality and structure of sporocarp) have received a comparatively large share of attention, but the conclusions even here are contradictory and unsatisfactory. Spore development and nuclear phenomena have been worked out to some extent in the Basidiomycetes and Discomycetes, Harper's work on one genus stands alone for the Perisporiaceæ, while nothing at all has yet appeared for the sphæriaceous Pyrenomycetes. Hence certain members of the last named group have been made the basis for the investigations of which the results are here presented.

METHODS.

Growing.—The material used for study was grown in artificial cultures and examined either in sections or by growing it upon glass slips immersed in nutrient media and transferring these slips directly to the stage of the microscope. The latter method served as a check on the former, since in sections the numerous cut ends of the mycelium are likely to lead to erroneous conclusions. As soon as pure cultures of the desired species were obtained by ordinary dilution methods, the spores were sown in

infusions of bean stems or mushrooms in Petri dishes. In these dishes had been placed carefully sterilized glass slips and cover-slips. The spores germinated sometimes upon the slips, sometimes floating free in the liquid, in which case the colonies rested upon the surface. Where they germinated on the slips, the mycelium adhered closely to the glass and made the process of fixing and staining much less difficult. On the other hand, the colonies which floated on the surface of the liquid seemed to take on more natural characteristics and they could be lifted out on a slide and prepared for the microscope. Many of the colonies thus grown developed mycelium in such abundance as to make observation very difficult. This was especially true of colonies grown in mushroom infusion, which seemed a most favorable medium, but it was also true that in these dense colonies the fruit was most abundant. The material for sectioning was grown in potato agar slightly acidulated to suppress bacteria.

Fixing and staining.—Slides bearing colonies were lifted carefully from the Petri dishes at different stages in their development and washed in water. Various methods of fixing and staining were tried. The most satisfactory was to fix with a cold 1 per cent. acetic saturate solution of mercuric chloride, stain with alum-eosin and mount in glycerine. The mercuric chloride fixes this material almost instantly and when washed off well with water leaves it in condition to take a clear decisive stain in the alum-eosin. This stain does not bring out nuclear structures with great distinctness, but cell walls come out sharp and clear. It stains the young mycelium and perithecia almost instantly, the latter taking a slightly deeper stain than the former. Good results were also obtained by staining with carm-alum the material fixed in this way. Ehrlich-Biondi, hæmatoxylin and fuchsin were also found fairly good. The efficiency of any stain was found to depend largely upon the age of the fungus. Young mycelial threads stain very readily, while the very mature ones refused the stain altogether.

Sectioning.—Small blocks of potato agar containing colo-

nies were cut out from the Petri dishes, dropped for a few minutes into the mercuric chloride and then allowed to wash in running water over night or at least for six to eight hours. In this process much of the agar was washed away, but the colonies were sufficiently compact to hold themselves together. They were then passed through successive alcohols, infiltrated and embedded in collodion, and cut with a microtome in sections from 6μ to 12μ thick. No other fixative tried seemed to leave the material so susceptible to stains as the mercuric chloride. Among others used may be mentioned chromic acid, Flemming's stronger solution, Hermann's solution, and Fish's picro-aceto-sublimate. The solutions of Flemming and Hermann were perhaps equally good when cleared with hydrogen peroxide, but without this additional trouble the sections so fixed were useless because of the discoloration of the protoplasm. With Fish's mixture some very good sections were prepared, but they were in no way superior to those fixed in the more simple corrosive sublimate.

In all the earlier part of the work the sections were stained with Mayer's carmalum. This produced no differentiation of nuclear structures, and much time was spent in experimenting with various stains and combinations of stains in the hope of getting a color differentiation. This was not accomplished, but in the later work upon nuclei Hermann's safranin-gentian-violet method as given by Zimmerman (17) was employed with very satisfactory results. Gjuarsin's methods as given in his article on Discomycetes were also found valuable.

The greater part of the observations were made with a Bausch and Lomb microscope, one-twelfth inch objective and one inch ocular, but for nuclear study this was found insufficient, and a Zeiss instrument with a one-twelfth inch objective and no. 8 ocular was employed.

TEICHOSPORA.

This fungus was found growing upon dead branches of oak in the vicinity of Ithaca, N. Y., late in November 1895. The

perithecia were then past maturity and many had discharged their spores. Closely associated with the perithecia were numerous pycnidia, appearing as much smaller rounded black bodies. A dilution culture was made in potato agar from the ascospores which were supposed to be free from the pycnospores. The ascospores soon germinated and camera lucida drawings of the germinating spores were made (*fig. 1*). Numerous colonies now appeared in the plates. They were circular, showing radiations from the center, and in concentric rings, a little later, the beginnings of perithecium like bodies appeared. These colonies were then transferred to infusions of bean stems in tubes where they continued to develop. Contrary to expectation, however, when the round black perithecia like bodies matured they contained not asci, but pycnospores. The pycnidia were rounded or oval in form, opening usually by two apertures (sometimes by more, sometimes by only one), and extruding their spores in the typical worm like manner. The spores are fusoid-elliptical, hyaline, continuous, about half the diameter of the mycelium. In the colonies the pycnidia appear first near the center and may be found in all stages of development along a radius of the colony, the youngest stages nearest the circumference. The beginnings of these structures are shown in *figs. 4* and *5*. No pycnospores had been observed in the agar plates in which the sowing was made, and since the ascospores were known to have germinated, the natural conclusion was that ascospores had produced pycnidia, but this conclusion was found to be doubtworthy later. Meantime, there had appeared in the bean tubes, associated with these pycnidia, a grayish white mycelium with erect branching hyphæ upon which were borne multiseptate conidia (*fig. 6*). The hyphæ were closely septate and the spores varied from the diameter of the hyphæ, when they were hyaline or yellowish in color, to twice that diameter as they became dark brown. Although these were normally borne on the tips of hyphæ, as true conidia, they sometimes appeared as intercalary growths in the mycelium. When these conidia were quite mature a sowing of them was made in potato agar. Colonies

soon began to form around the spores, but whether the growth began from the germination of the spores or from attached fragments of mycelium was not determined. As only pycnidia and conidia were produced from these sowings, the cultures were abandoned. Sowings of pycnospores were next tried, but in several successive generations only pycnidia and gonidia appeared and there seemed no hope of reproducing the ascigerous fruit. This failure of the ascospores to reproduce themselves even in alternate generations seemed unnatural and another culture was attempted from the original material. This had meantime been kept in the laboratory and had become old and dry and resisted all efforts to make it germinate. On January 25, 1896, some fresh material was collected by Professor Atkinson and from this ascospores were obtained which germinated readily in potato agar. Certain spores in the plates were marked and watched carefully until February 1, when they had attained distinctive characters and the numerous other colonies in the plates could be safely and certainly identified as the same. They appeared to the naked eye as dark spots with a light radiating fringe. With a carefully sterilized scalpel transfers of these colonies were made to bean stems in tubes. Ten such tubes were prepared. The transfers were made in a close culture-room and with the utmost precaution to avoid contamination. There was no doubt that these colonies grew from the ascospores, but probably none of these colonies developed. For a long time no growth appeared. Finally, in two tubes, a few pycnidia were found, and in one or two other tubes non-related forms appeared. In the plates from which the ten transfers had been made there were found (Feb. 3) a few colonies much in advance of those which had been traced from the ascospores, but very strongly resembling the pycnidia bearing colonies of previous cultures. These, however, contained the beginnings of perithecia in some of which asci had already developed. These colonies had apparently grown from fragments of mycelium or of the perithecia. They were removed piecemeal at short intervals of time (about three hours) and fixed and embedded for sectioning. It

was hoped by this means to get all the successive stages of development (see account of sections below). Transfers of these larger colonies were also made to the bean stems in tubes, where they continued to develop perithecia and March 5 a culture was made from the ascospores contained in these perithecia. These spores, although full grown, were still hyaline in color, but they possessed unusual vigor. The colonies grew very rapidly and developed to maturity. Perithecia only were produced and the same was true in a third generation of this series. Another culture was now made from the pycnidia and conidia but again they produced only their own kind. This failure to connect the ascospores with the other forms led to the conclusion that they are probably distinct forms. The ascospores obtained from the oak in the fall germinated but failed to produce thrifty colonies, probably because they had lost vitality or because they were not readily adaptable to artificial conditions. Those sown in the first cultures very probably germinated and then died, as did those transferred to the ten tubes. The pycnidia found in these first cultures no doubt originated from pycnospores which were associated with the asci but overlooked in making the culture. This might easily have been the case, since the pycnospores are very small and scarcely distinguishable in potato agar. The study of the pycnidia and conidia has been included here because of interesting points of resemblance in the development of the pycnidium and the perithecium, and also because in nature the two forms are found so intimately associated. Indeed their connection is not disproved, although it has been impossible to establish artificially a life cycle including the three forms.

The ascigerous colonies which were finally obtained started, as has been said, from fragments of the mycelium or perithecia, and the ascospores thus obtained, being fresh and vigorous and perhaps also somewhat inured to conditions, germinated readily and reproduced themselves. This is further evidenced by the fact that during the winter two or three attempts were made to get cultures of the following species from herbarium specimens: *T. trimorpha*, *T. fusispora*, *T. aspera*, and *T. nitida*. The first named

species had been in the laboratory but a few months and its spores germinated but developed no further. The others were older and refused even to germinate.

Attention was now confined to the ascigerous colonies of the species in hand, and with methods previously described the following observations were made. The mycelium is composed of septate threads, each cell of which contains several nuclei. With alum-eosin and carmalum stains, the nuclei are distinguishable merely as round points stained more deeply than the cytoplasm and lying at or near the center of a circular clear space. In the mycelium nothing more minute than this could be determined in regard to the structure of the nucleus. None of various stains tried succeeded in differentiating any elements. Any stain which affected the nucleus also attacked the protoplasm of the cell. The nuclear membrane was usually sharply defined as a dividing wall between the clear circle and surrounding cytoplasm.

At various intervals in the mycelium certain cells were found which were more or less swollen (*fig.* 7). The protoplasm in these was more dense than in the adjoining cells as shown by the deeper staining. This over-staining made observations upon nuclei in these cells very uncertain, but the cells proved to be the beginnings of perithecia. The first dividing wall is thrown across parallel to the septa which delimit the original cell (*fig.* 8). Each of the two daughter cells then divides by a wall perpendicular to this, forming a four-celled spherical body. As this grows, further divisions occur somewhat irregularly (*figs.* 9 to 11) until we have a body consisting of a solid mass of irregular cells as yet without any differentiation. Sometimes at this stage the young perithecium is surrounded by a single layer of mycelial threads which have arisen in the neighborhood of the original cell and interwoven themselves, forming a sort of wall for the perithecium. The wall proper in this species is not more than a single layer of cells thick and in many cases it seems to be formed by the thickening of the cell walls of the outer layer of the spherical mass, without the assistance of any surrounding filaments. It does not become dark and hard until the perithe-

cium is nearly or quite full grown. When the growing perithecium has attained a little more than half the normal diameter of the mature fruit there may be seen at its center an oval sac containing a single nucleus and filled with densely granular protoplasm which stains very deeply. This sac is simply a swollen cell of the interior of the sphere. Simultaneously with its appearance a part of the loose parenchymatous tissue surrounding it breaks down so that the young ascus lies in a more or less disorganized, gelatinous mass. Very soon the other asci appear one by one. In many cases the sections showed two large ovoid sacs lying side by side and almost filling the interior of the perithecium. The later asci developed as the growth of the sphere made room for them.

Earlier investigators have attempted to find the connection between the origin of the asci and a supposed fertilized archicarp (see Woronin, De Bary, and Hartig, *l. c.*). In this case there exists no probability of a process of fertilization. In many cases the entire sporocarp may be traced from a single cell around which no other filaments are present even to take part in the construction of the wall, and in cases where extraneous filaments are applied to the archicarp it happens after several divisions of the original cell have taken place. If we consider the single swollen cell in the mycelium as the mother cell of the entire perithecium it is a question of some interest what determines which of the daughter cells become asci and which are disorganized. Harper (5) finds in Sphærotheca a structure consisting of from five to seven cells, arising directly from the fertilized archicarp, and a certain one of these containing two nuclei and giving origin to the ascus. We might consider the entire cellular structure of the fruit of Teichospora homologous with this five to seven-celled growth in Sphærotheca, in which case we should expect to find in the former certain binucleated cells functioning as mother cells of the asci. This may indeed be true but the observations so far made do not warrant such a statement. It is only in exceptional cases that the nuclei in these cells can be distinguished at all, on account of the density of the proto-

plasm and consequent deep staining. In the earlier stages, when the structure consists of two to four cells, faint outlines of nuclei can sometimes be seen, and in one or two cases division was suspected, but no positive statements are warranted regarding the nuclei in the perithecium prior to the formation of the ascus. As soon as the ascus is differentiated, however, the nucleus becomes very distinct. No fusion has been observed, and in the youngest stages each ascus contained one large clearly defined nucleus. This nucleus is much larger than those found in the mycelium, but is otherwise similar.

That some process of nuclear division takes place is apparent. As before stated, the young ascus contains a single nucleus. This has been called by De Bary the "primary nucleus." It does not, however, as he says, lie always in the end of the ascus. Its position is variable, but it is found oftenest near the center of the ascus and in a bed of very dense protoplasm. Its diameter is often equal to half that of the ascus. Neither does this primary nucleus disappear and eight daughter nuclei appear simultaneously, as has been stated. All the successive stages of division have not been observed, but an ascus containing two nuclei was found, and another with eight. In the latter case they were arranged in pairs, the two largest having moved a greater distance apart than any other pair (*fig. 19*). This, with the further evidence about to be given, was considered sufficient proof that the eight nuclei are provided by successive bipartitions of the primary nucleus. On this point the ascus next to be described was indirectly very instructive (*fig. 20*). In its protoplasmic content could be discovered, by careful focusing, the outlines of eight spores. The protoplasm in the spores was more dense than that surrounding them, and hence they were stained more deeply. The two spores nearest the base of the ascus contained one nucleus each and were not divided. A third spore contained two nuclei, lying near the ends of the spore, but no dividing wall had yet appeared. Four other spores contained two nuclei each, situated at various distances from a central septum. This septum was unstained, appearing like an open

space in the protoplasm, but was more distinct than the outlines of the spores. The eighth spore of this ascus was in such a position that only part of its outline could be seen. In another ascus (*fig. 21*) there were found eight well defined spores, the walls being now distinct. Seven of these were divided by single central transverse walls into two cells each. Each cell contained a single nucleus. The eighth spore was bent upon itself in such a way as to conceal one end and only one nucleus was visible in it. There can be little doubt, however, that another was present in the concealed end, and it may be asserted safely that at this stage the ascus contains uniseptate spores having a nucleus in each compartment. The septa in this case had been formed soon after the first nuclear division in the spore. In preparations from material slightly more mature, spores were found in which the number of nuclei varied from two to ten, corresponding in number usually to the number of cells in the spore. An exception to this occurred in the case of one entire perithecium (*fig. 16*). Here each spore contained from four to ten nuclei but no dividing walls could be seen. Either the staining was at fault, which seems improbable, or the formation of the wall does not always follow immediately upon nuclear division, but takes place instead after all the successive bipartitions of the nucleus are complete.

The foregoing evidence, together with the fact that the nuclei occur constantly in pairs throughout the mycelium as well as the fruit, seemed conclusive proof that nuclear division does take place, but for a long time no details of the process of division could be discovered. Later, cases were found in which the hyaline court had become elliptical in outline and contained sometimes one eccentric dark spot, and sometimes one at each end of the ellipse. These at first seemed very similar, and the nucleus in this condition corresponds closely with Trow's (14) figures of what he calls "fragmentation" in the nucleus of Saprolegnia. If his theory be correct then the central stained spot is probably a chromatin plate, and by simple fragmentation furnishes a half plate for each daughter nucleus, and this grows quickly to full size. This must be based upon the assumption that the two

stained bodies at the foci of the ellipse are similar in structure. This indeed seemed at first to be true, since no differentiation resulted from the use of the ordinary differential stains. On the other hand, it seemed quite as possible and more probable that the original dark spot of the nucleus is the nucleolus. In a resting condition this alone is visible, but preparatory to division the nucleolus moves off to an eccentric position, the hyaline court elongates, and the chromosomes accumulate to form the second chromatin mass in the opposite end of the ellipse. With more exact staining methods and greater magnification this possibility proved to be the fact. The resting nucleus contains a large nucleolus in the center of the cell sap. In this sap in some cases can be seen an indistinct linin network of very fine threads (*fig. 27*). Preparatory to division the nucleolus becomes eccentric and the circular space elongates. The linin now becomes more distinct, the threads having grown thicker at certain points (*fig. 28*). In the next stage small deeply stained chromatin bodies (not more than four have been counted) appear at the end of the ellipse opposite the nucleolus, arranged in what might be the equatorial plane of a spindle, but no spindle threads have been distinguishable in any case. At the poles of this plane were two small rounded bodies which were probably centrospheres, although no radiating striæ could be seen (*fig. 29*). Again, two groups of chromosomes, of four each, were found near the positions previously occupied by the centrospheres (*fig. 30*). By this time the nuclear membrane had become partially dissolved. The nucleolus was now vacuolated and less deeply stained and lay partially out in the surrounding cytoplasm. The cell sap was still present but constituted a somewhat distorted mass, and in it were the outlines of two new hyaloplasmic circles each surrounding a group of daughter chromosomes. In the next stage the ascus contained two new nuclei, somewhat smaller than the original but having the same structure (*fig. 31*). No secondary division was observed. Upon these cases, which have been illustrated from camera lucida drawings, may be based the conclusion that the nuclei in these asci divide karyo-

kinetically. The failure to discover the spindle may have been due to inadequate staining or insufficient magnification. In other essential details the process agrees with Harper's description of karyokinesis in the Discomycetes. If the details seem incomplete it must be remembered that the largest nuclei measured only five or six micromillimeters in diameter, and minute structure was extremely difficult to determine with the instruments available.

The general sequence in the process of the development of the ascigerous fruit of Teichospora may now be summarized as follows:

1. A single cell of the mycelium by successive divisions and growth forms a solid sphere of parenchymatous tissue.

2. Certain of the interior cells of this tissue become enlarged and differentiated into asci.

3. Each ascus contains at first a single large nucleus, which by successive karyokinetic divisions furnishes a single smaller nucleus for each compartment of the multiseptate spores.

TEICHOSPORELLA.

An undescribed species of Teichosporella, found by Miss Stoneman on oak and studied in a similar way, was found to develop in almost exactly the same manner as the Teichospora. So similar are the steps that it seems unnecessary to repeat the description in detail. A few figures are given showing the early stages of the perithecia (*figs. 7a*, *10a*, *11a*) as confirmatory evidence of the rather unusual way in which the sporocarps in this group arise, and also to call attention to a slight peculiarity which may have some significance in the question of phylogenetic relationships. This peculiarity is shown at *x* in *figs. 10a* and *11a*. It consists of a protuberance in the region of the archicarp, which by its shape and position suggests the possibility that it may be a degenerate rudiment of an antheridium. This feature is quite constant, and in general appearance not unlike the antheridia found later in Ceratostoma. If this explanation of its presence be accepted, then this genus furnishes a connecting link between

forms having more or less complete sexuality and the Teichospora where all trace of an antheridium has disappeared.

Teichosporella exhibits another peculiarity in the development of the ascus. Instead of an evenly granular protoplasmic content filling the ascus from the time of its origin, there exists here, at first, an apparently empty sac formed, not by the swelling of a cell as in Teichospora, but as a papillate and then somewhat inflated outgrowth from a cell. Into this sac the protoplasm seems to push its way through a comparatively narrow opening at the base. The process may be understood best by reference to *figs. 22* to *26*, in which successive stages of the process are shown. The same conditions were found in the living material, hence it could not have been due to the action of reagents. The appearance may be due to an abnormal swelling of the ascus wall which recedes with its growth, or to the presence in the young ascus of a non-chromatic plasma or cell sap. At certain stages a zone of this same colorless sap may be seen enveloping the spores (*figs. 25* and *26*) after the ascus itself has become filled with the normal protoplasm.

The nuclear processes in this species agree, so far as studied, with those in Teichospora, but the work on this form was not repeated with the better stains and higher magnification used in the later work on Teichospora.

The development of the sporocarp as above given for these two genera is indeed widely different from any process heretofore described for the perithecia of any of the Pyrenomycetes. It is interesting to note, however, that it corresponds very closely with what has frequently been described as the normal course of development of pycnidia. This and the apparent loss of sexuality here suggests that these may be more degenerate forms than some others, and further that extreme degeneracy leads to the production of pycnidia only, these last named fruit forms being merely reduced perithecia.

CERATOSTOMA BREVIROSTRE.

This fungus was found growing upon decayed mushrooms in a garden near the university. In artificial cultures it produced

fruit so rapidly and in such abundance that it seemed a specially favorable subject for developmental study. The large dark brown spores in germinating extruded an endospore through a tiny pore at the more pointed end of the spore. The endospore contained a large vacuole and many nuclei, and from it arose one or more germ tubes (*fig. 32*). These germinating spores have been drawn large to show the nuclei, which are very distinct, occurring usually in pairs, but so small as to require great magnification in order to be seen at all. Spores sown in potato agar or upon bean stems produced colonies of whitish mycelium, sending out from the center strong primary radiating filaments which become plumose at their tips.

The colonies spread flat upon the substratum, and when grown in tubes the radiating threads pushed far out upon the glass, bearing fruit at some distance from the medium upon which the spore had germinated. This fact proved of great advantage in the later microscopic study, since the thin weft of mycelium grown on slides in Petri dishes could be easily transferred to the stage of the microscope and afforded excellent opportunity for study of the material in the best possible condition. The mycelium was comparatively scanty and no conidia or pycnidia appeared at any time during the many successive cultures that were made. The necks of the perithecia furnished a noticeable example of heliotropism. Those grown in tubes standing in boxes, and hence lighted only from above, turned strongly upward. A tube was then wrapped in dark paper, leaving only about an inch at the bottom exposed to the light, and suspended by a string. In this the necks turned sharply downward. Those grown in plates inclined always towards the window.

The first microscopic study was directed specially toward determining, if possible, whether there exists here any sexual organs such as have been described by Woronin (9) for the closely related genus Sordaria. For this purpose the fungus was grown upon bean stems and prepared for sectioning in collodion. Various methods of fixing and staining were tried, but nothing con-

cerning the earliest stages of the perithecia could be discovered. Colonies grown in agar were next tried. These were fixed, hardened, and embedded in much the same manner as those grown on stems. This also proved unsatisfactory. The cut ends of the mycelial threads were deceptive, and although some very suggestive features appeared no positive conclusions could be drawn. An attempt was next made to study the colonies in cell-cultures and in the agar plates in which they grew. The latter proved more nearly successful than any of the former methods, but still much was concealed by the cloudiness of the agar and the fact that few colonies grew close enough to the surface to be focused upon with the higher power objectives. Finally the plan of growing on glass slips was adopted and gave the desired results. Slides bearing colonies were removed from the dishes at successive short intervals and treated as before described. The greatest caution was necessary to keep the colonies *in situ*. Unless they had grown very close to the slide they were speedily tumbled into a tangled, shapeless mass by the action of the fluids. The colonies which grew floating in the liquids required endless patience in preparation, but in this delicate material, when it was successfully prepared and mounted in glycerine, the long sought beginnings of perithecia were finally discovered. They occurred in such abundance as to leave no doubt of their function. They were noticeable first as deeply stained spots scattered thickly through the mycelium and varying in size from a little more than the diameter of the mycelium to one-fourth that of the diameter of a normally mature perithecium. This amount of variation in size could be seen usually in a single preparation, and so slight and uniform were the gradations from the larger to the smaller that there remained no doubt of the two being identical structures. The smallest of these bodies consisted of a short swollen branch arising from a primary branch of the mycelium and immediately becoming curved. From this short slightly curved branch (*fig. 33*) to the several coiled type (*fig. 34*) all intermediate stages were found. The size and shape varied greatly, and from the various assortment only the more typical forms

were chosen for illustration. In most cases, an antheridial branch in contact with this curved structure (which was evidently the archicarp) could be plainly seen. This was a slender branch arising usually, though not always, from another filament and applying itself to the archicarp. In some instances the antheridia traveled comparatively long distances to reach the archicarps, and some were found coiled once or twice upon themselves in their courses. In most cases the two gametes were so interwoven that they could not be exactly traced, but in two instances unmistakable fusion of antheridium with archicarp was discovered (*figs. 35* and *36*). In both cases the antheridial branch is applied just at the tip of the female organ and the walls of both at the point of contact are dissolved. Many nuclei are usually present in both gametes, but they have not been seen fusing. The antheridium does not lose its protoplasmic content. It is usually less deeply stained than the archicarp, but this is also true before the two organs meet, and means probably only that the latter is richer in protoplasm than the former. The antheridium, moreover, is not always present. Numerous instances occurred, as shown in *fig. 33*, where the archicarp seemed to be developing without fertilization. There is, of course, no positive proof that these non-fertilized cells would ever produce asci, but the evidence given by the older stages of these points very conclusively toward the existence of parthenogenesis. Other peculiarities such as those shown in *figs. 37* and *38* were very interesting. In *fig. 38* the antheridium, while certainly present, has turned entirely away from the female branch, while the latter has continued to coil very much beyond the limit at which fusion usually takes place. *Fig. 33* also has become septate in accordance with the normal plan of development after fertilization. *Fig. 37* shows a tendency in another direction. Here the archicarp, having failed of fertilization, has been produced into a vegetative filament in a manner suggestive of what the writer (19) finds occurs at times in Vaucheria. These vegetative outgrowths were comparatively rare, but still were of sufficiently frequent occurrence to demand attention. They might indeed

be caused quite as easily by insufficient nutrition as by lack of fertilization.

About the time that fusion of the two gametes occurs, in normal cases numerous branches arise in the neighborhood of the young fruit and become intimately interwoven around the sexual organs to form the wall. For a time the swollen archicarp can be seen in the center of this knotted mass of sterile filaments, but as the wall thickens and the threads of which it is composed become more closely septate this interesting structure is lost from sight. For further steps in the process it is therefore necessary to refer to sections. To get very early stages it was necessary to fix the growing material before any sign of color appeared on the outside of the tiny rounded bodies which were just becoming visible in the mycelium. The rate of growth varied so much that no definite age could be established as the proper one for sectioning, and repeated trials were made before sections containing the desired information were secured. By means of a long series of observations it was at last determined that in the stage just preceding the origin of the asci, the perithecium consists of a spheroidal mass of cells of three kinds. First is an outer layer two or three cells thick of thick-walled, nearly isodiametric cells, made up of the sterile hyphæ which envelop the young sexual organs. Inside this is a layer, two to three cells in thickness, of tabular cells which appear to have been laterally compressed by growth from within. These two have evidently been formed from the enveloping hyphæ. The center of the sphere is entirely filled with loose, spongy tissue composed of parenchymatous cells, well filled with protoplasm. These cells show no differentiation of form, and nothing exists to indicate where the asci will originate save that in certain sections a small group of these cells, lying about midway between the center and circumference of the spongy tissue on one side, takes a slightly deeper strain. Even in the very young stages no sign of the sexual organs imbedded in the spongy tissue could be found. It is at this point that De Bary and others have been obliged to leave gaps in their records of perithecial devel-

opment. De Bary (1) says of Xylaria that the hyphal coil, or "Woronin's hypha" as it has been termed, seems to disappear before the formation of the asci begins. Hartig (10), in his study of Rosellinia, states that the asci seem to spring from a certain gelatinized zone, but whether they originate in the sexual apparatus or in the *Wandparenchyma* he is unable to determine. Woronin (9), too, fails to trace the exact origin of the ascus in Sordaria or to find the connection between it and the "hyphal coil." That a structure so prominent as this sexual apparatus, if it persists throughout the later development, should elude the notice of so many observers is quite incredible. It seems quite as improbable that this fertilized archicarp should fail to play any part in the production of the ascospores Harper (5) finds in Sphærotheca that the oosphere grows out into a branch of five to seven cells, from a certain one of which the ascus arises. The existence of the sterile cells of this branch suggests a solution of the problem in the true Pyrenomycetes, which has already been partially verified in the case of Teichospora, and is further confirmed by what is found here. Before the enveloping weft of threads, which are to constitute the wall, becomes too dense it may be observed that the coiled archicarp is becoming septate. Even in very young stages this septation has gone so far that in section the interior of the perithecium appears as a homogeneous mass of cells in which the outline of the coil cannot be distinguished. It now seems logical to conclude that this entire mass of parenchyma which forms the interior of the immature perithecium is formed by successive cell divisions in the archicarp. Instead of the five to seven-celled branch, giving rise to one ascus in Sphærotheca, we have here an indefinite number of cells giving rise to a variable number of asci. This is strictly analogous with the process in Teichospora, which was more easily traced because of the absence there of enveloping threads. It is in no way contradictory to the observations of Woronin, De Bary, and Hartig, though it fills in the gap which they have left open. It is true that only a comparatively very small number of these cells give birth to asci and the question as to what determines the fertile cells is still

open. Here, as in Teichospora, no binucleated cells were discovered, but it was also true, as in the former case, that either because of the density of the protoplasm in these cells, or because of some fault in manipulation, no nuclei at all were discovered in any of these cells. As has been said, the only differentiation is a slightly deeper stain in a certain group of cells, the position of which corresponds with that from which the asci arise. It may be argued that only this group of deeply staining cells are formed by division of the archicarp, but if this were true we should certainly expect in some cases to be able to trace the outlines of the fertile hypha. Moreover this latter theory would fail to account for the existence of the sterile cells. The marked difference in structure between these cells and those of the wall make it highly improbable that both arise from the enveloping hyphæ. Then, too, when the sections become mutilated by the rolling of the collodion or by rough handling it is a noticeable fact that this central tissue breaks out intact, leaving the inner surface of the wall quite smooth. This theory of development tends also to harmonize what first appeared to be a very peculiar condition in Teichospora. The archicarp in Teichospora is not fertilized and is not specialized in form, but the further processes of development are essentially homologous. There remains only to prove that the mother cells of the asci contain two nuclei which fuse before entering the ascus in order to establish a line of development analogous not only to that described for Sphærotheca but also to what is found in many cases in the Florideæ. This intervention of vegetative tissue between fertilization and the production of spores, provides a sporophyte phase in the life cycle of these fungi which has not hitherto been known.

From the deeply stained group of cells in the central parenchyma the asci arise as papillate outgrowths, densely filled with protoplasm and staining much more deeply than the surrounding tissue. Their bases lie close together and the asci, which are long narrow-cylindrical and very numerous curve upward toward the ostiolum. Their protoplasm is densely gran-

ular and usually somewhat vacuolated. As they grow they become slightly constricted at the base. Certain ones advance more rapidly than others, so that in a single perithecium all stages of development may frequently be seen. The "bouquet" of variously sized asci lies embedded in the loose surrounding parenchyma, a part of which persists until the asci are quite mature. A distinct layer of this tissue is present between the bases of the asci and the basal part of the wall. In the center of the perithecium, between the asci, these vegetative cells become disorganized and probably furnish nourishment for the growing asci.

Meantime the wall has also undergone some changes. With the growth of the asci the entire sporocarp enlarges, the cells of the outer wall become thicker walled and darker, while those of the inner wall undergo still more lateral compression. At a point opposite the base the ostiolum begins to appear. Its beginning is marked by a slight protuberance, beneath which first the cells of the inner then those of the outer wall begin to separate schizogenetically. The ends of these elongated inner wall cells after separation round off and look like filaments converging toward the canal of the ostiolum. As the neck increases in length these filaments also lengthen and extend up into the neck canal parallel with its sides. The growth of the ostiolum continues after the development within is quite complete. Indeed the asci often are broken down, leaving the spores free within the cavity before the neck reaches its normal length.

The nuclei of the mycelium have already been noticed. They seem essentially the same in structure as those found in Teichospora, consisting, so far as could be seen, of a nucleolus in a clear circular court. The fact that no linin has been seen in the nuclei of the mycelium is probably due to the small size of these nuclei and the fact that they are seen through the wall of the mycelium. In the ascus the nuclei of Ceratostoma are smaller than in Teichospora and fewer details were made out. The drawings were reproduced free-hand, as those made from the camera lucida were too small for satisfactory illustration. In

these nuclei there may be seen a linin network of very fine threads within the hyaline court (*figs. 40* and *41*). The first step toward division is the elongation of the court. The nucleus then becomes eccentric and the chromatin collects in masses on the threads. Then the network disappears. In the next stage that could be found the chromosomes had evidently divided and were arranged in two groups at some distance apart (*figs. 40* to *42*.) No centrosomes or spindle figure could be certainly distinguished, though in one or two cases something very like a spindle figure was faintly visible. Many of the intermediate stages are wanting. What becomes of the nucleolus cannot be certainly stated, but enough of the steps have been observed to warrant the conclusion that there is here a karyokinetic division, the more minute details of which might be detected by higher magnification. The stages found are illustrated in *fig. 40*, nos. 1–4.

HYPOCOPRA.

As Teichosporella served to confirm the observations made on Teichospora, so Hypocopra served as a check upon Ceratostoma. The species studied was obtained from dung and separated by transferring with a sterilized needle first to dung infusions and then to bean stems, where it grew and fruited abundantly. From the bean it adapted itself readily to different media. The germination of the spores (*fig. 42*) was studied by transferring small quantities of the agar in which they grew to slides, where they were stained with Mayer's carmalum and mounted in glycerine. They did not, as in Ceratostoma, extrude the endospore. The protoplasm exudes through a tiny pore at the end of the spore and pushes out into a germ tube. The division and growth of the nuclei takes place with startling rapidity. Before the germ tube attains half the length of the spore, from four to eight nuclei have appeared. The colonies of mycelium grown in agar are characterized by the appearance of the first perithecia in a definite ring at a short radius from the center of the colony. The sexual organs are essentially similar to those of Ceratostoma. Some of the more typical forms are

shown in *figs. 43* and *44*. The hyphal coil may be traced for a longer time here than in the other genus. In *figs. 45* and *47* it is shown somewhat straightened out and several times septate. For want of time the study of this form was not carried through to the end, but enough observations were made to furnish convincing proof that the course of development is essentially the same as in Ceratostoma. The process is somewhat more complicated than in Teichospora, and may be summarized as follows:

1. The spores upon germination send out polynucleated mycelial threads which become septate, branch, and form circular colonies.

2. Upon the mycelium are borne short thick branches which become curved, or sometimes several times coiled, and function as archicarps.

3. Near these archicarps are usually found long slender branches, the antheridia.

4. The antheridia intertwine with the archicarp, their tips meet and fuse.

5. The archicarps in some cases appear to develop without fertilization.

6. The archicarp by growth and division furnishes the cells which make up the interior of the perithecium.

7. From certain of these cells of the interior the asci arise.

8. In each young ascus there is a single primary nucleus.

9. The primary nucleus divides karyokinetically and the daughter nuclei in the same manner, to furnish a nucleus for each spore.

10. Nuclear division probably continues within the spore after the formation of the spore wall.

11. The wall of the perithecium is formed from surrounding filaments.

The evidence furnished by the foregoing investigations tends o corroborate the theory of De Bary that marked analogies exist between the higher fungi and the Florideæ. In the Pyrenomy-

cetes we may expect to find sexual organs and sexual processes in different stages of degeneracy. In Ceratostoma we have distinct sexual organs, but the first sometimes develops without the fertilization of the archicarp. In Teichosporella there remains only a possible rudiment of an antheridium, while in Teichospora this organ has entirely disappeared.

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EXPLANATION OF PLATES XIV–XVI.

Figs. 1, 2, 3, germinating spores of Teichospora.

Figs. 4 and 5, young stages of pycnidia.

Fig. 6, conidia.

Figs. 7 to 12, successive stages in early development of perithecium of Teichospora; *7a*, *10a*, *11a*, early stages of perithecia in Teichosporella.

Fig. 13, later stage of perithecium of Teichospora in which certain cells have begun to differentiate into asci and contain primary nuclei.

Fig. 14, perithecium showing young asci.

Fig. 15, part of a perithecium with several asci each containing primary nucleus.

Fig. 16, fragment of a perithecium in which all the nuclear divisions in the spores seem to have occurred without the formation of dividing walls.

Figs. 17 to 21, successive stages in development of the ascus in Teichospora, showing nuclear phenomena.

Figs. 22 to 26, stages in development of ascus in Teichosporella. Magnification not sufficient to show nuclei.

Figs. 27 to 31, successive stages in nuclear division in Teichospora.

Fig. 32, germinating ascospores of Ceratostoma brevirostre.

Figs. 33 and 34, archicarps or beginnings of perithecia without attendant antheridia.

Figs. 35 and 36, archicarps with antheridia attached, showing fusion.

Fig. 37, unfertilized archicarp produced into vegetative filament.

Fig. 38, archicarp and functionless antheridium.

Fig. 39, young perithecium in which the archicarp is still visible in optical section.

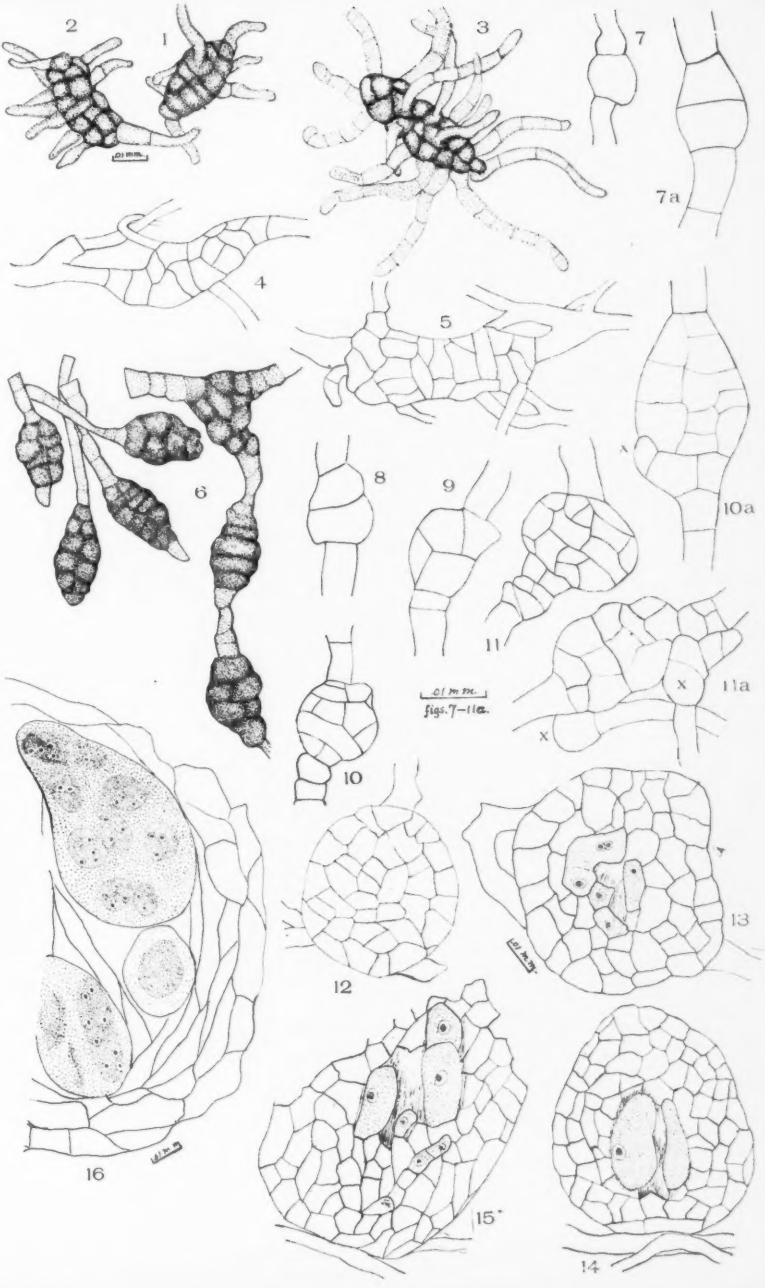
Fig. 40, fragment from the base of perithecium in which asci have begun to appear; *w. p.*, wall-parenchyma; *t*, tabular layer or inner wall. Asci are numbered to indicate successive stages in nuclear division.

Fig. 41, further development of asci.

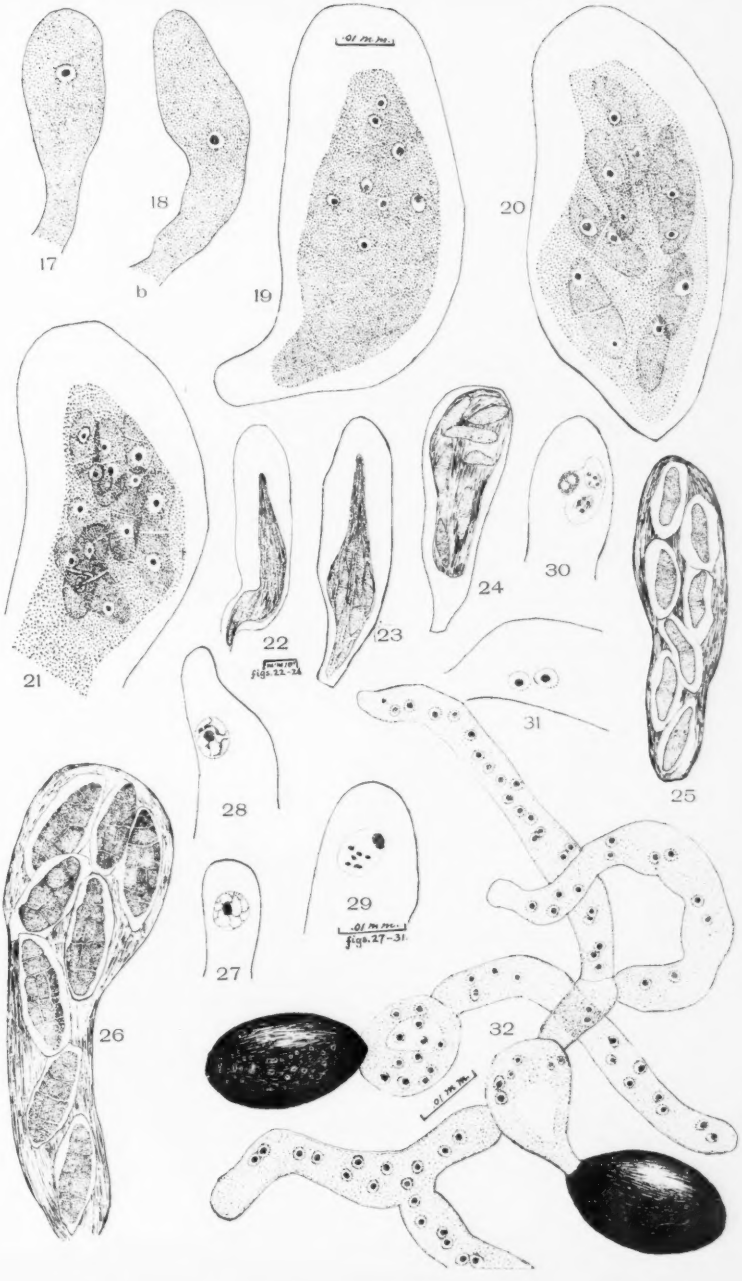
Fig. 42, germinating spores of Hypocopra.

Figs. 43 and 44, archicarps of Hypocopra with antheridia present.

Figs. 45 to 47, young perithecia in optical section showing enclosed archicarp.



NICHOLS on PYRENOMYCETES.



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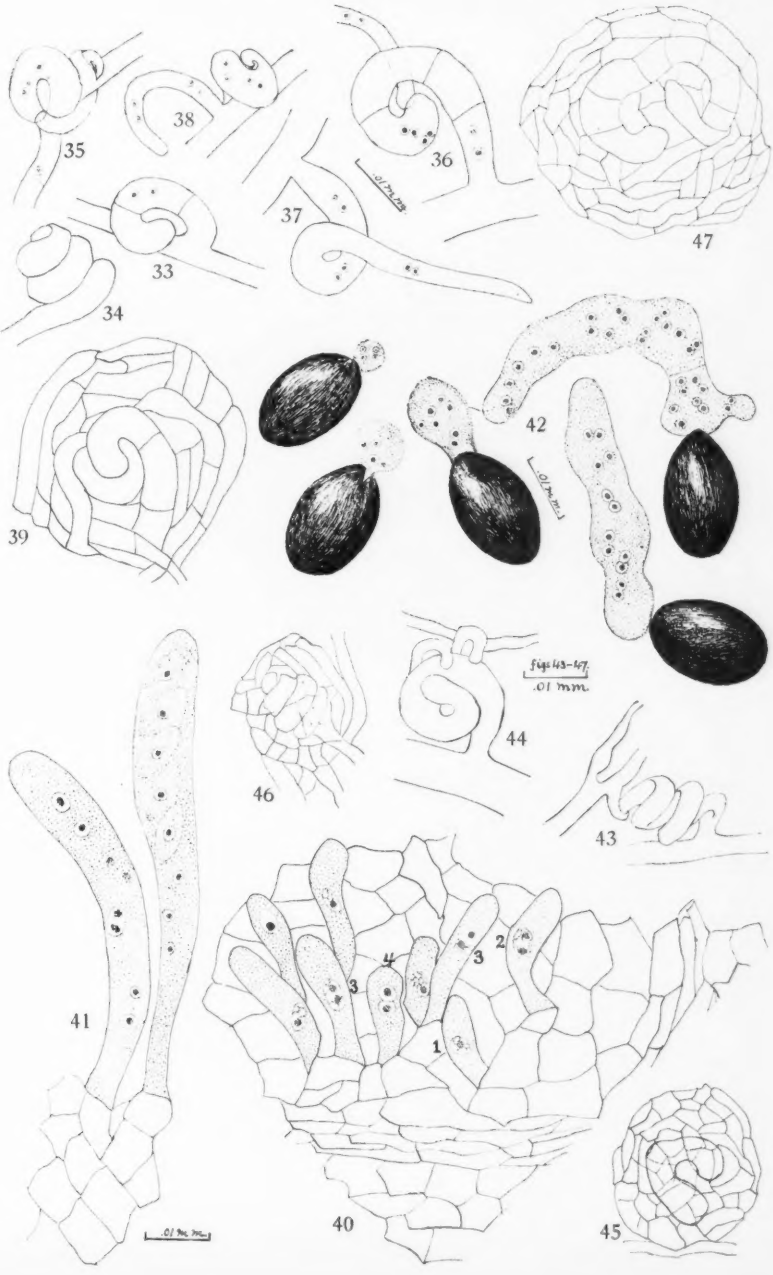


NICHOLS on PYRENOMYCETES.

BRIEFER ARTICLES.

THE ORGANS OF ATTACHMENT IN BOTRYTIS VULGARIS.

(WITH PLATE XVII)

WHILE engaged during the present year with a series of cultures of the common greenhouse fungus familiarly known as *Botrytis vulgaris*, the attention of the writer was attracted by the facility with which conidiophores and organs of attachment mutually replace each other as the result of changes of external conditions. The interest of such facts from a theoretical standpoint makes it seem worth while to record the following observations which were conducted under the direction of Professor V. M. Spalding.

Preliminary artificial cultures were prepared, each in a drop of malt solution on a glass slide. The first two, although as nearly alike as possible, showed very different results. One in the course of three days was completely covered with conidiophores (*fig. 1*), while in the other these were almost entirely absent, a development of peculiar cellular masses, the so-called organs of attachment, having taken place instead (*fig. 6*). The phenomenon suggested at once a relation of some kind between the organs of attachment and the conidiophores.

Other cultures were then prepared on slides, both plane and with concave centers, in test tubes, flasks, Petri dishes, etc., with the result that whenever the hyphæ came in contact with the hard surface of the culture dishes, these peculiar bodies appeared, and whenever the hyphæ grew upward without contact, conidiophores developed.

In order to determine with certainty whether or not the formation of conidiophores and organs of attachment could be controlled at will, the following cultures were prepared: In a drop of malt solution, placed on each of several slides with concave centers, were sown one or more conidia, and over the drop was suspended, by means of strips of damp blotting paper, a coverslip (2×1^{in}). These cultures were then transferred to moist chambers until wanted. The slips were placed at various heights, if close to the drop ($1-2^{mm}$ above) in two or three days the entire overhanging surface was covered by organs of attachment, while

conidiophores were formed only where hyphæ escaped at the sides and grew freely upward. When the slips were placed at greater heights ($3-7^{mm}$), a greater development of conidiophores followed with a corresponding decrease of organs of attachment.

Next slips of mica were perforated with the point of a fine needle and placed at various heights as in the above cultures, with the result that organs of attachment were formed where hyphæ came in contact with the overhanging surface, and conidiophores where they grew opposite or through the perforations. The fungus seemed to be entirely indifferent to light and gravity, and the formation of organs of attachment to be conditioned solely by the contact of hyphæ with a hard surface.

The development of the conidiophores and organs of attachment throws still farther light on their relation to each other. For the study of the developing conidiophores rather old perianths of *Lilium candidum* were inoculated with conidia from a clean culture and placed in moist chambers. In the course of three or four days the entire substratum was covered by the fleecy mycelium. From time to time this was examined until all the stages of development were obtained. Briefly stated they are as follows: Strong erect hyphæ, rich in protoplasm, branch two or three times, and from these branches repeated secondary divisions are sent out (*fig. 2*). Ultimately the apices of the branches swell, and peg like protuberances (*fig. 2 a*) appear which rapidly increase in size and number so that in a comparatively short time mature ovoid conidia (*fig. 2 b*) are developed from them. This formation of conidia was in progress at 3 o'clock P.M. April 14. Thus, with respect to the time of spore formation, this fungus differs from the one described by Klein.[1]

For the study of the successive stages of development of the organs of attachment, the following agar-agar cultures were prepared: malt solution, 2 per cent.; malt solution, 3 per cent.; geranium decoction, 2 per cent.; potato decoction, 2 per cent.; grape sugar, 2 per cent. In all of these media the fungus grew luxuriantly, producing organs of attachment when contact was possible, and conidiophores when this was impossible. The actual development of the organs of attachment was traced from a single conidium sown in 2 per cent. potato agar medium, as follows:

[1] Ueber die Ursachen der ausschliesslich-nächtlichen Sporenbildung von Botrytis cinerea. *Bot. Zeit.* **43**: 6. 1885.

After germination of the conidium, the fungus grew rapidly, branching in every direction, and whenever an upright hypha came in contact with the cover of the shallow Petri dish (5.5^{mm} inside depth) repeated branching took place as in case of the conidiophores. As the organs increased in size the branches became shorter so that ultimately they were of no larger size than ordinary conidia of this species. The successive stages in the development are represented in *figs. 3–6*. *Fig. 3* shows the condition of things at 3:15 P. M.; *fig. 4* at 4:15 P. M.; *fig. 5* at 5:07; and *fig. 6* at 6 P. M. of the same day. After this the branching became so rapid and the organ so complex that accurate drawings were out of the question.

When we compare the successive stages in the development of the conidiophores and organs of attachment, we find them essentially alike in origin and mode of branching, but ultimately in the case of the developing conidiophore the tips swell and peg like projections are sent out which grow and become mature conidia, while in case of the organ of attachment the branching continues irregularly and indefinitely. Farther, when conidia are sown in nutrient solutions germ tubes are sent out, and when a nutrient solution is supplied to one of the organs of attachment each ultimate division sends out a tube resembling a germ tube. In both cases these hyphæ branch and develop into a complex mycelial mass. The resemblance therefore is a physiological as well as a morphological one.

The connection between conidiophores and organs of attachment is farther emphasized by the intermediate forms found in almost every culture. *Fig. 7*, drawn accurately, as the others are, with the camera lucida, represents a prostrate branch with a young organ of attachment at *b*, and short, erect branches bearing conidia at *a*. It was a common occurrence for a hypha to bear conidia in successive clusters until it came in contact with the cover of the Petri dish, when it produced an organ of attachment.

Up to this time the organs of attachment were studied under artificial conditions, but later attention was directed toward their formation and significance in nature. Leaves in connection with vigorously growing plants were tied in such a manner as to be from 2–4^{mm} above each other, conidia having been sown previously, either in a small drop of water or malt solution, on the under leaf. The plants were then placed in an atmosphere saturated with moisture, under a large bell jar. In no case was penetration observed where conidia were sown in

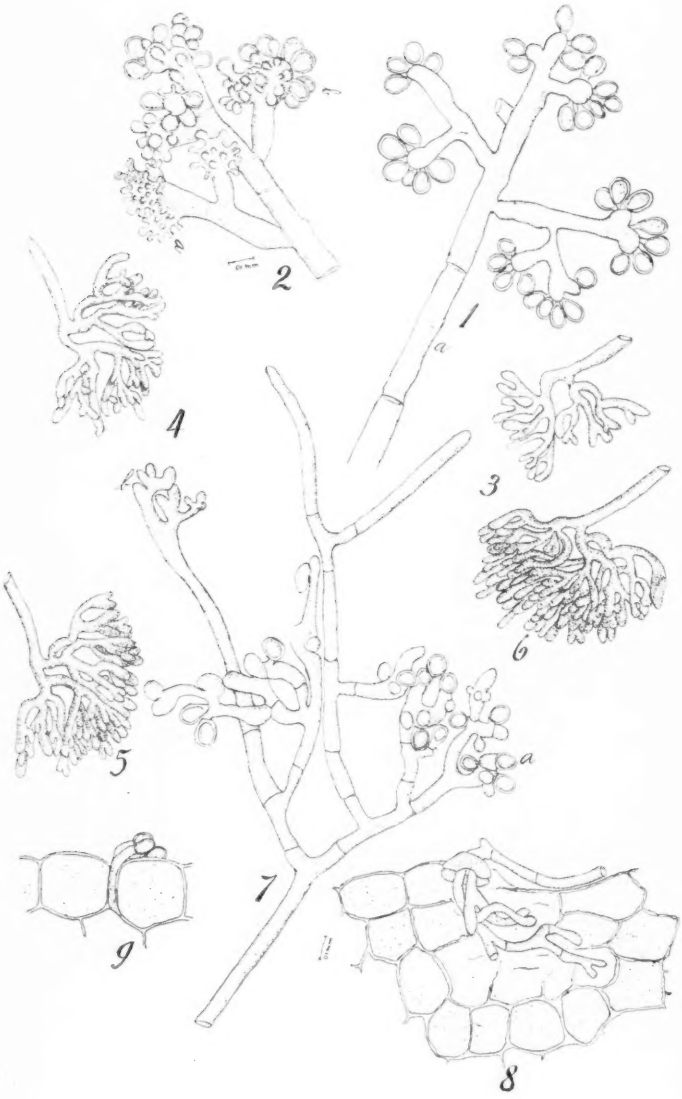
a drop of water simply, but where they were started in nutritive media, whether malt solution or a very small dead insect, in two or three days the fungus had penetrated both leaves, the one on which the conidia were sown as well as the one from 2–4^{mm} above it. Organs of attachment formed freely on the overhanging surface here as in the artificial cultures already described, while on the lower surface they were rarely met with. Whenever organs of attachment had thus formed tubes were sent out from them into the host in the manner described by former writers. But that the fungus is not confined to this means of entering the host was seen in sections of a leaf on which conidia had been sown in a drop of malt solution four days previous to sectioning. Here contact with other leaves was impossible, so that the erect hyphæ bore conidia, while the prostrate ones ran over the surface of the leaf until a stoma was found for entrance (*fig. 8*). Again, some of the conidia produced by this mycelium had germinated and sent germ-tubes directly into the leaf between the lamellæ of two adjacent cells (*fig. 9*). In the latter case the tissue of the leaf was probably weakened by the presence of the fungus in some other part, since after entrance the tissue is rapidly consumed.

From the foregoing experiments, and others not recorded, the writer concludes that with this fungus the formation of organs of attachment is determined by external conditions which may be artificially produced by placing in proximity to the hyphæ a hard surface for contact, or they may be met with in nature where plants are crowded together so that the leaves of different plants lap over each other; that when this condition is not present conidiophores will be developed instead of organs of attachment; that in general conidiophores and organs of attachment are both physiologically and morphologically equivalent; that biologically the fungus makes use of the organs of attachment to penetrate a neighboring leaf, or it has the alternative, after starting saprophytically, of entering the host either through the stomata or by sending germ tubes directly into the tissue.

The literature of *Botrytis vulgaris* and allied forms is considerable, yet comparatively little has been said concerning the morphology of the organs of attachment. Brefeld[2] classes them, on account of their manner of attaching themselves to a substratum, with other "Haft organen," while De Bary[3] throws more light on their biological rela-

[2] Schimmelpilze **4**: 112.

[3] Bot. Zeit. **44**: 382, 412. 1886.



HORN on BOTRYTIS.

tion to the host, saying that they are first formed as the result of a mechanical stimulus, and that by means of a poisonous secretion they injure the tissue, draw nourishment from it, and as soon as this is obtained send out branches which for the most part do not penetrate the host directly, but spread over the surface surrounding the organ. Later Marshall Ward[4] described their development, and speaks of them as being "of the same morphological nature as those figured by Brefeld in *Peziza sclerotiorum*, and explained by De Bary subsequently as organs of attachment," while J. E. Humphrey[5] describes them as compact cellular masses which cling closely to the surface with which they are in contact, but that their real significance is not yet understood. — MARGARETHA E. C. HORN, *University of Michigan*.

EXPLANATION OF PLATE XVII.

Fig. 1. A branch of an upright hypha bearing conidia.

Fig. 2. Young developing conidiophore with small projections at *a*, and mature conidia at *b*.

Figs. 3–6. Successive stages of a developing organ of attachment.

Fig. 7. A prostrate branch bearing mature conidia at *a* and a young organ of attachment at *b*.

Fig. 8. Hypha entering a leaf of geranium through the stoma.

Fig. 9. Germ tubes penetrating the host between the lamellæ of two adjacent cells.

NEW WEST VIRGINIA LICHENS.

Lecidea Virginiensis Calk. & Nyl., sp. nov.—Thallus glaucescens tenuis laevigatus rimulosus citrino-flavus; apothecia fusca aut nigra convexiuscula immarginata latit. circ. 0.5^{mm}, intus medio sordida; spora oblonga incoloris, long. 0.009–0.012, crass. $0.004–6^{mm}$; epithecium et pars media hypothecii dilute fuscescens. In toto gelatina hymenialis coerulescens, dein theca vinose rubescens.

E stirpe videtur *Lecidea sanguineo-atra*, prope *Lecideam delineatam* Nyl.

On sandstone rock under the drip of a wet cliff. West Virginia, near Nuttallburg, L. W. Nuttall coll. no. 1779. *Flora of West Virginia*, Millsp. & Nutt., 181.

[4] Ann. Bot. **2**: 327. 1888.

[5] Ninth Ann. Rept. 1891 and Tenth Ann. Rept. 1892. Mass. Agr. Exp. Station.

Lecidea Nuttallii Calk. & Nyl., sp. nov.—Apothecia nigra parva; epithecium impressum; spora fusca oviformis 1-septata, long. 0.014–16, crass. 0.005–6^{mm}; hypothecium fuscum. In toto gelatina hymenialis vinose rubescens.

Super thallum *Ricasolia sublævis* Nyl. West Virginia, near Nuttallburg, L. W. Nuttall coll. no 1781. *Flora of West Virginia*, Millsp. & Nutt., 181.

Arthonia aleuromela Nyl., sp. nov.—Thallus albus subfarinaceus chrysogonidicus tenuissimus; apothecia subrotundata vel oblonga, prominula, latit. 0.4–0.5^{mm}; spora oblongo-oviformis parte inferiore attenuata, 1-septata, long. 0.010–11, crass. 0.003^{mm}. In toto gelatina hymenialis cœrulescens, dein obscurata.

Thallus detritus subaureus, CaCl vix reagens. Gonidia chroolepoidea fulvescentia.

On bark of *Quercus sp.* West Virginia, near Nuttallburg, alt. 2000 feet, L. W. Nuttall coll. no 1182. *Flora of West Virginia*, Millsp. and Nutt., 182.

Lecanora deplanans Nyl., sp. nov.—Thallus glauco-cinerascens tenuis areolato-rimosus determinatus; apothecia badio-rufescens (satis diluta) innata subconcaviuscula, latit. 0.5–0.7^{mm}; spora ellipsoidea, long. 0.015–16, crass. 0.009–1.010^{mm}; epithecium inspersum. In toto gelatina hymenialis fulvo-rubescens.

Videtur species e stirpe *Lecanora cervina*, spermatiis ellipsoideis.

On rocks in bed of creek. West Virginia, Short Creek, alt. 1300 feet, L. W. Nuttall coll. no. 1126. *Flora of West Virginia*, Millsp. & Nutt. 178.—C. F. MILLSPAUGH, *Field Columbian Museum, Chicago*, and L. W. NUTTALL.

EDITORIALS.

Botanic Gardens

THE SUBJECT of botanic gardens is happily coming into prominence in the United States. The recent address of Dr. Britton at Buffalo has put into our hands a succinct account of what has been done abroad and what has been begun in this country. The showing for America is better than was expected, and the promise of the immediate future seems to be very large. The two essential features of a botanic garden, popular education and botanical research, have been lost sight of for the most part in the numerous so-called gardens of many cities. Unless under scientific control they become merely places of cheap display, pleasant enough but not specially instructive, and certainly offering no facilities for research. Probably the only possible condition in which a botanic garden can be made to fulfill its real mission is to develop it in connection with a university, but if left to the university alone it will seldom command sufficient income to become largely effective. If a combination can be made between a university and a city, as in the case of the New York Botanical Garden, the largest results are possible. The growing demands of botanical science have brought every university face to face with the problem of a botanic garden, and it seems likely that the solution of this problem in America lies in the combination of university and municipality.

THIS COMBINATION may not be so difficult as some suppose. Almost every municipality has one or more parks in various stages of cultivation. In most cases, if under high cultivation, the same monotonous succession of a few common plants in beds of conventional form appears. It is astonishing to note the limited range of plants ordinarily selected for parks, to the exclusion of the hosts of forms awaiting cultivation and of far more interest even to the park frequenting public. In such a case an arrangement might be made between the park commissioners and the university by which a certain portion of the park area should be under the control of the university as to the plants to be cultivated. The advantage to the commissioners

would be the securing of expert advice in reference to the plants adapted to interest and instruct the public; the advantage to the university would be the securing of abundant illustrative material without the cost of its maintenance. Certainly the parks need to command a certain amount of botanical knowledge, and the universities are equally in need of a larger contact with plants in their various relations.

ASIDE FROM the ordinary uses by the university of what is styled illustrative material, any large control of planting would secure the possibility of experimental work in various biological lines without interfering with the legitimate uses of a park. Problems connected with heredity would be perfectly feasible, such as otherwise would demand the large outlay connected with the equipment of a special experiment station. In case of too great distance between the park and university a small "field laboratory" would make possible such work as would suffer by transportation. It is often said that most of our universities have about them wild areas that are a sufficient botanic garden. This is true in case botanical instruction and research is to go no farther than it has in this country, but it certainly is not true if it is to advance in the directions indicated by the signs of the times. Botanical laboratories, to properly maintain themselves as centers of current instruction and research, are compelled to provide for plants in masses, grouped with a purpose, and subject to control.

Duplication of Publication

THE PUBLICATION of articles upon the same research in different journals under the same or slightly different titles is a growing custom and an evil one. When a subject is a many sided one, with relations to several branches of science, there is justification for such duplication. The paper in the August number of the GAZETTE, by Kahlenberg and True, for example, is a most suggestive one, not only in chemistry, but also in medicine, bacteriology, and physiology. The editors felt that its botanical bearing was sufficient to justify its publication in the GAZETTE, though it is to appear afterward in full in another form and though a brief abstract of it had already been printed.

BUT WE WERE not aware that Mr. Maxwell's paper upon the growth of banana leaves, which was printed in our June number, was to be republished in the *Botanisches Centralblatt* about July 1, and we doubt

very much whether the editor of that journal knew that it had already been published. Its importance did not justify republication. The paper was received by the GAZETTE through Dr. Goodale, whom the author requested to have it published in an American journal, with no intimation that he was sending another copy elsewhere. This is not the first time that the GAZETTE has been imposed upon in this way, which speaks better for the faith of the editors in botanists than for the good faith of the authors. Certainly common honesty requires that authors give editors an opportunity to refuse papers which they expect to duplicate thus.

IF WE ARE not mistaken, the publication of one paper stating fully the nature and results of a research ought to end publication until further research has been made and new results reached. Some eminent botanists have in late years followed a different course, and have worked over the same studies into three or four different papers in different journals. But if results are of real value one adequate publication is all they need to receive recognition and all that ought to be unloaded upon already burdened bibliographers. We go so far as to say that the "preliminary paper" with its half prepared diagnoses or ill-digested generalizations is an unmixed evil and ought to be suppressed by botanical opinion. We are glad to join *Natural Science* in its vigorous opposition to such makeshift methods.

OPEN LETTERS.

SOME RECENT PAPERS ON NOMENCLATURE.

To the Editors of the Botanical Gazette:—Although the Rochester rules have given American botanists some tangible guide in nomenclature, and the recommendations of the Harvard Memorandum have provided the believers in letting bad enough alone with some means of mitigating the chaotic conditions to which they have become accustomed, it does not seem advisable to cease all agitation upon the subject of nomenclature. It may be true that the time so spent would be better spent in other lines. But if the enormous waste of time which will eventually be entailed by the establishment of four or five distinct nomenclatures in as many botanical centers may be obviated by a slight expenditure of time now, surely such use of it will not be entirely vain.

My only purpose in this note is to call attention to some recent publications of that indefatigable and zealous reformer, Dr. Otto Kuntze, who is endeavoring to secure a competent international congress and through such a congress an international nomenclature. The botanical world, as Dr. Kuntze points out, now has at least four more or less distinct systems of nomenclature. Each is gaining currency in the regions under its peculiar influence, and each, he asserts, is obstinately maintained by its promoters, who in consequence are unwilling to take any active interest in securing an international code. The world has the basis of such a code in the Parisian laws, which are to a greater or less extent at the foundation of each of the present systems. But the interpretation and application of the Paris code is a matter of generaldis agreement, and its insufficiency in its present form is unquestioned.

In 1895 Dr. Kuntze published an article entitled "*Les Besoins de la Nomenclature botanique*" in which, after pointing out the danger to nomenclature arising from the existence of local codes or systems at Berlin, at Kew, and in America, he puts forward nine propositions for a congress to be held at Paris in 1900. Dr. Kuntze's idea of what such a congress should be deserves more attention than it has received. He does not believe in a gathering of men fresh from other studies which, without having given the matter more than passing attention, shall proceed to settle all disputed points at once. To anyone who has had experience with the numberless unexpected and complicated problems which a settlement of the subject must dispose of, if it is to be a settlement at all, it is apparent that a great deal of preliminary work must be done in the way of testing the application of various rules suggested so that those who are to decide upon them may do so intelligently, and in ascertaining just what are the defects to be remedied and what are the dis-

urbing elements in our present nomenclature so that the settlement may reach all of them. Dr. Kuntze believes that this work can hardly be done in a thorough manner between now and 1900.

In a circular to the Société Botanique de France, published in March of the present year, Dr. Kuntze reiterates the importance of preparation for the projected congress. He says: "It (the congress) cannot honestly inscribe in its order of the day the revision of the Parisian code without a necessary international preparation lasting three years at least." When it is remembered that Dr. Kuntze's scheme of such a congress involves the putting out of a "Nomenclator Plantarum omnium," it will be seen that the importance of preparation is not exaggerated. Dr. Kuntze possesses qualifications both of experience and otherwise that point him out for the compiler of such a nomenclator. It would be a great pity to throw away the opportunity of securing his services in constructing one upon the lines of an international code. He will doubtless go on with his work of preparing it in any case.

In the *Oesterreichische Botanische Zeitschrift* for May of this year, in the *Journal de Botanique* of May 16, in the *Bulletin de l'Herbier Boissier* for July, and the *Journal of Botany* of the same month, are articles by Dr. Kuntze, in each of which he urges the necessity of a congress and points out the danger of the present state of things. It seems proper to call attention to these articles if only because of the sincerity and admirable zeal of their author.

The repeated protests of Dr. Kuntze against the establishment of four or five distinct nomenclatures in as many places are not to be treated lightly. One great object of nomenclature is to secure international currency for plant names. If we are not to have this, we may as well throw Latin nomenclature over and use the vernacular. The condition of things in which "sage brush" gets into European works as "Salvia" is not greatly bettered by one in which four or five nomenclators will have to be used and cross references made in order to be sure what a given binomial refers to. I have followed and shall continue to follow the Rochester rules because I see no other rules available for American botanists, and because I prefer rules to caprice as a guide, whether I entirely agree with the rules or not. But if American botanists are to be content with legislation for their own needs and are to remain indifferent to or even to hinder international action, will Dr. Kuntze's taunt that we are anarchists be wholly unwarranted?

In Mr. Erwin F. Smith's "Protest" against the check-list, he says: "What we need is the speedy convening of a representative international botanical congress, which shall amend the Paris code . . . . and shall settle once for all certain disputed interpretations of this code." Those of us who have been unwilling to abide in anarchy till such a congress is convened should not be less active in urging competent international action than our more conservative brothers.— ROSCOE POUND, *Lincoln, Nebraska.*

CURRENT LITERATURE.

MINOR NOTICES.

THE PLANTS of various regions in the state of New York have formed the basis for good catalogues. The latest of these is that issued by the Rochester Academy of Science.[1] It is the region studied from 1836 to 1867 by Dr. Chester Dewey, and occupied ever since by an aggressive race of botanists. It is more than a catalogue, as all the features of the area which have any relation to plant distribution are discussed, and interesting comparisons are made with the Cayuga and Buffalo floras. The native species of phanerogams enumerated are 948 in number, the introduced species 250: but including well marked varieties the phanerogamic flora as now constituted is made up of 1,314 distinct forms.—J. M. C.

THE LAST contribution from the National Herbarium[2] contains a variety of material. A. S. Hitchcock reports upon a collection of plants of 193 numbers made by C. H. Thompson in southwestern Kansas in 1893. F. V. Coville discusses *Crepis occidentalis* and its allies, seven species being described and figured, four of which are new. J. N. Rose reports upon a collection made by Mr. Frank Tweedy in 1893 in the Big Horn mountains of Wyoming. John M. Coulter and J. N. Rose describe and figure a new umbelliferous genus, Leibergia, from Idaho and Washington. Alfred Cogniaux describes and figures Roseanthus, a new cucurbitaceous genus from Mexico, dedicated to Mr. J. N. Rose. As this number completes the volume a very full index is given.—J. M. C.

A FORM of plant association which he calls *protrophy* has been described at length by Dr. Arthur Minks in a recent volume from the press of Friedländer.[3] In 1892 a new *Lebensgemeinschaft*, with the name syntrophy, was described by the same author, which, however, does not seem to have

[1] BECKWITH, FLORENCE, and MACAULEY, MARY E., assisted by Joseph B. Fuller. —Plants of Monroe county, New York, and adjacent territory. Large 8vo. pp. 150. Published by the Rochester Academy of Science, June 1896. $1.00.

[2] Contributions from the U. S. National Herbarium 3: 537–612. 1896.

[3] MINKS, ARTHUR:—Die Protrophie, eine neue Lebensgemeinschaft, in ihren auffälligsten Erscheinungen. 8vo. pp. viii+247. Berlin: R. Friedländer & Sohn. 1896.

impressed morphologists as sufficiently important to be included in modern texts. The reader will find the present work a mine, from which we doubt whether he can take out any ore of value. Certain it is that what he does get he will have to dig for amid the intricacies of involved sentences and the obscurity of a technical terminology that will daunt him from the very beginning. As nearly as we can understand Dr. Minks' protrophy is an association between two species of lichens; one, the protroph, being unable at the beginning of its existence to nourish itself and needing therefore to fasten upon the body of the other independent species which precedes it, and to utilize this so far as necessary as a protection and support until it also can become independent. Protrophy is thus a special case of syntrophy, in which this dependent relation of the syntroph upon the other species is lifelong.

Readers who wish a fuller summary of the work will find a preliminary paper under the same title as the work in the *Oesterreichische botanische Zeitschrift* for February and March of this year.—C. R. B.

AN ACCOUNT of the history, types of variation and cultivation of the chrysanthemum has been published as an independent pamphlet by Henry L. de Vilmorin,[4] the well known horticulturist. The paper contains nothing new, but is an interesting description of the flower which has achieved a popularity never equaled.—C. R. B.

A WORK quite similar to Willkomm's for the Iberian peninsula[5] is the paper by Diels reprinted from Engler's *Botanischer Jahrbücher* on the "plant biology" of New Zealand.[6] Islands, of course, offer the best limited regions for a study of plant distribution, especially if it be a mountainous one like New Zealand. Two general regions are recognizable in this island, viz., forest and alpine. Under each of these Diels discusses the plant formations, with full attention to the ecology of the plants under consideration, treating such topics as water absorption and storage, assimilation, dorsiventrality, etc The peculiar structural adaptations of several species are figured. A page of rosette plants from the alpine region is especially striking. Finally the vegetation of the remaining islands of the same faunistic zone (Lord Howe, Norfolk, Kermadec, the Chatham group and the Antarctic islands) is discussed. The paper closes with a section showing how the present flora of New Zealand is the outcome of the geological history of the island.—C. R. B.

[4] DE VILMORIN, HENRY L.:—Le chrysanthème; histoire, physiologie, et culture en France et a l'étranger. Imp. 8vo. pp. 28. figs. 10. Paris: the author. 1896.

[5] See BOTANICAL GAZETTE **22**: 62. 1896.

[6] DIELS, L.:—Vegetations-Biologie von Neu Seeland. Separat-Abdruck aus Engler's Bot. Jahrb. **22**: 202–300. Pl. 3, figs. 7. 1896.

FAMILIAR TREES AND THEIR LEAVES[7] is the name of a popular book by F. Schuyler Mathews, in which are described over 200 trees of the eastern half of the United States, including not only native but commonly planted species. These descriptions are not at all technical, yet give the characteristics of the tree, its general habit and distribution, and point out the features by which it is separated from similar ones. The illustrations of leaves and generally also of fruits, which accompany the descriptions, will enable one to identify most of the common trees. The difficult task of rendering *texture* in the black and white sketch has not been accomplished by the author-artist, but the outlines are accurate. Less than one-third have been drawn from living specimens and others from herbarium material.

The *raison d'être* of the brief introduction by Professor Bailey, except for the value of his name on the title page, does not appear. The book is certainly to be warmly commended to those, to use Professor Bailey's words, who desire to know the tree as an entirety and to have some knowledge of its kinship and names, and who simply want an introduction to the trees which they meet.—C. R. B.

IN THE REPORT of the botanical department of the State Agricultural college of Michigan for 1895, Dr. W. J. Beal describes the botanic garden designed and planted by him upon the college grounds. A list of the species growing therein and a map of the garden on a scale of about 50 feet to the inch are given.—C. R. B.

MISS MINNIE REED has adapted Barnes' *Key to North American Mosses* to the 165 species found in Kansas, prefixing to it an account of the structure of the mosses (which is not without a number of errors) and adding nine well drawn but poorly printed plates, illustrating thirty-six species. Each generic key is also followed by an account of the geographical distribution within the state. The list, only recently received, is dated by the author June 1893 and is reprinted from the *Transactions of the Kansas Academy of Science for 1893–4*, pp. 152–199.—C. R. B.

RECENT BULLETINS from the Department of Agriculture are these: The Chief of the Division of Vegetable Physiology and Pathology, Mr. Galloway, writes, in the *Experiment Station Record*,[8] a suggestive though brief paper on

[7] MATHEWS, F. SCHUYLER: Familiar trees and their leaves described and illustrated, with over 200 drawings by the author and an introduction by Professor L. H. Bailey of Cornell University. 12mo pp. x + 320. New York: D. Appleton & Co. 1896. $1.75.

[8] Reprinted as a separate, and issued by the office of experiment stations as a bulletin.

the "lines of investigation that might be undertaken by experiment stations." These words need emphasis: "One of the serious drawbacks to advanced research work is this very matter of continued duplication of work already being done by other stations and the running along in the same old grooves year after year. We cannot hope to have this difficulty remedied, however, until there is some attempt at unification of purpose or specialization on the part of stations."

Dr. Walter H. Evans writes of "Copper sulfate and germination,"[9] that fungicide being commonly used to prevent smut by soaking the seed. Many contradictory observations are recorded regarding the effect upon germination of soaked seed. He finds that 0.5 and 1 per cent. solutions do no serious injury in 1–2 hours, which is adequate to kill smut spores, and that much stronger solutions can be used if seed are planted at once. Some of his statements need revision in the light of Kahlenberg and True's work on the "Toxic action of dissolved salts."[10]

Mr. Jared G. Smith has brought together[11] brief untechnical descriptions of the "Fodder and forage plants exclusive of the grasses," including 200 species, both native and exotic, illustrated by fifty-six figures. It forms a very convenient reference list.—C. R. B.

A SECOND EDITION of the catalogue of the plants of Los Angeles county, California,[12] is said on the cover to be a reprint from the *Proceedings of the Southern California Academy of Sciences* but gives no other indication of being anything but an independent pamphlet. Dr. A. Davidson, who prepared the first list in October 1892, is also the author of this. In this county, embracing 4,000 square miles, 100 miles of seacoast, the San Gabriel mountains reaching 6,000 feet, a part of the Mojave desert and the islands San Clemente and Catalina, 934 species and varieties of spermatophytes and 27 of pteridophytes are known. A second part, listing the remaining cryptogams is promised, some day.—C. R. B.

IN A PAMPHLET of eighteen pages with the curious title *Labrador*, published at Munich under date of July 1896, Dr. F. Arnold has given a list of the lichens collected in late years on the east coast of Labrador by Mr. J. W. Eckfeldt and Rev. Arthur Waghorne. One hundred and twenty-seven species are enumerated.—C. R. B.

[9] Bulletin no. 10, Division Veg. Phys. and Path. 1896.

[10] BOT. GAZ. **22**: 81. 1896.

[11] Bulletin no. 2, Division of Agrostology. 1896.

[12] DAVIDSON, ANSTRUTHER: Catalogue of the plants of Los Angeles county. Part 1 — Phænogamia. Reprint from the *Proceedings of the Southern California Academy of Sciences*, 8vo. pp. iv + 36, *pl. 1*. 35 cents.

NOTES FOR STUDENTS.

CARDOT has described[1] five new species of Fontinalis from North America: *F. patula* (§ TROPIDOPHYLLÆ), from Vancouver near Victoria, collected by Macoun; *F. Missourica* (§ HETEROPHYLLÆ), from Benton county, Missouri, collected by Demetrio; *F. Dalecarlica Macounii* (§ LEPIDOPHYLLÆ), from Athabasca Lake, collected by Macoun; *F. Waghornei*, from Trinity Bay, New Harbor, and Witter's Bay, Newfoundland, collected by Waghorne; *F. MacMillani* (§ MALACOPHYLLÆ), from northern Minnesota, collected by MacMillan.—C. R. B.

STUDENTS of forest distribution will be interested in Professor T. H. MacBride's discussion of forest distribution in Iowa.[2] He shows that all the students of the forest problems in his state have been right, but only partially so. His own conclusions, which combine views which seemed to be in conflict, are as follows: (1) the immediate agent in the limitation and distribution of Iowa forests was fire; (2) the sweep of fire was determined by a modicum of moisture and by the presence of fuel upon the ground; (3) the drift being especially adapted to gramineous vegetation furnished fuel in such amount as to prevent the development of tree seedlings, while the loess, using the term in a broad sense, less suited to gramineous species, furnished less fuel, hence gave to tree seedlings on loess regions opportunity to rise; (4) special localities, as swamps, alluvial flood-plains, etc., present special cases and require special explanations.

THE FOLLOWING items are of taxonomic interest: Dr. John K. Small[3] has prepared a synopsis of the North American species of Ilysanthes, recognizing six species, one of which is new. Mr. Eugene P. Bicknell[4] recognizes two species in the well known Scrophularia of the eastern states, the segregated one being called *S. leporella*. Mr. H. N. Ridley[5] has described a new genus of Commelinaceæ from the Malay Peninsula, under the name Spatholirion. M. A. Franchet has described eight new species of Saxifraga[6] and ten new species of Sedum[7] from China, and proposes a new section[8] (Xyphosandra) of Parnassia, in which the very acuminate connective is produced far beyond the loculi, giving to the anther the appearance of a dagger. Miss Alice Eastwood[9] has described ten new species from southeastern Utah. Mr. J. G. Baker[10] has published an illustrated synopsis of the genus Brodiæa, as

[1] Revue Bryologique — : 67. 1896.
[2] Reprint from Proc. Iowa Acad. Sci. **3**: 96–101. 1895.
[3] Bull. Torr. Bot. Club **23**: 296. 1896.
[4] *Ibid.* **23**: 314. 1896.
[5] Jour. Bot. **34**: 329. 1896.
[6] Jour. de Botanique **10**: 261. 1896.
[7] *Ibid.* **10**: 284. 1896.
[8] *Ibid.* **10**: 267. 1896.
[9] Proc. Calif. Acad. Sci. II. **6**: 270. 1896.
[10] Gard. Chron. **20**: 213 and 238. 1896.

defined in Bentham and Hooker's *Genera Plantarum*. American plants recently figured, and with full descriptive text, are *Clematis Addisonii*,[11] *Lonicera hirsuta*,[12] *L. hirsuta* × *Sullivantii*,[12] *Aquilegia Jonesii*,[13] *Rhododendron Vaseyi*.[14]

Dr. B. L. Robinson, in a recent discussion[15] of the fruit of Tropidocarpum, calls attention to its great variability, and its consequent uselessness for taxonomic purposes, a fact which militates strongly against certain proposed species. Aside from the taxonomic features of the discussion the fact of greatest general interest is the occurrence of the internal capsule which frequently appears in what is known as the *capparideum* type of capsule. This internal capsule is variable in size, "from the merest obscure rudiment to a capsule half the length of the outer one." The outer capsule is always 3 or 4-valved, and the inner one always 2-valved, and when well developed contains two seeds, "which mature in just the same way as those in the surrounding capsule." The embryo also is apparently perfect, and the capsule regularly dehisces. As Dr. Robinson suggests, the fertilization of these innermost ovules is a very interesting problem. If they are reached by pollen tubes, these tubes must penetrate two styles. The inner capsules are usually axial in position, but sometimes arise near the base of the outer capsule. The author suggests that these inner capsules "represent a second whorl of carpellary leaves." Similar internal capsules have been noted by Peyritsch in *Draba alpina*.— J. M. C.

Hugo de Vries[16] has convinced himself by a long series of cultures that a large part of the teratological anomalies in plants are in their nature hereditary. His already known observations on the hereditary nature of fasciation and torsion are now followed by a discussion of adhesions and cohesions, or symphyses.

Having transplanted from a wheat field to his garden some individuals of *Hypochæris glabra* showing adhesions, he found the second generation with about 9 per cent. of similar adhesions, and the seventh with 64 per cent. A similar selection of *Helianthus annuus* with united cotyledons produced in the third generation plants showing 76, 81, and 89 per cent. of syncotylous embryos.

To these De Vries adds a host of similar facts from cultures and many observations of the repetition of teratological variations upon shrubs and trees. All, he thinks, point to the hereditary nature of the phenomena.

This heredity, he adds, sometimes appears "lateral," *i. e.*, it shows itself

[11] Gard. and For. **9**: 324. 1896.
[12] Gard. and For. **9**: 344. 1896.
[13] Gard. and For. **9**: 365. 1896.
[14] Gard. Chron. **20**: 71. 1896.
[15] Erythea **4**: 109. 1896.
[16] Botanisch Jaarboek **7**: 129–197. 1895.

in lateral branches of the genealogical tree, as in clover. It is hard, too, to get rid of it. The adhesions may skip an entire generation in annuals and reappear in the next, just as in perennials they may skip a year. The manifestation of the property depends to a high degree upon external conditions.

All the facts show that the heredity of adhesions is ordinarily latent, manifesting itself only occasionally.

The fact of heredity obliges us to suppose for the symphyses material carriers (pangens) in the protoplasts. But neither the number nor the influence of these appear to be ordinarily great enough to assure more than an occasional appearance of the anomalies. A concurrence of very favorable conditions seems always to be necessary to their manifestation, at least unless they have been fixed and accumulated by selection.—C. R. B.

MR. GEORGE MASSEE has made an exhaustive study of the genus Coprinus,[17] recognizing 165 species, 34 of which are credited to the United States and 20 of them peculiar to it. The evolution of form in the Agaricineæ is represented as proceeding from such primitive types as Marasmius, etc., in which "the pileus is sessile or stemless and fixed by its back to the substratum, the gills being uppermost and consequently entirely unprotected from the earliest stage of development." From this primitive type of structure there are three leading lines of departure: (1) turning the hymenium downwards; (2) the acquisition of a central stem; (3) the freedom of the gills from the stem. The Agaricineæ do not form a single group showing the above sequence, but are broken up into four series, each running through the lines of development indicated. These four series are characterized by the color of the spores (black, brown, pink or salmon, white), the Melanosporæ being the oldest and the Leucosporæ being the youngest. The chief biological feature of Coprinus is the deliquescence of the gills at maturity into a liquid which drips to the ground, carrying the mature spores along with it. This primitive and relatively imperfect mode of spore-dissemination, as compared with the minute, dry, wind borne spores of the other Agaricineæ, indicates that in Coprinus we have the remnant of a primitive group of fungi from which have descended the entire modern group of Agaricineæ with wind borne spores; and which can be traced back to the still more primitive subterranean fungi which are the common ancestors of the entire group of the Basidiomycetes. Evidences of the antiquity of Coprinus are seen in the world wide distribution of the genus, and the limited area occupied by species. Of the modern agarics the Melanosporæ are most closely allied to Coprinus, being directly derived from it, and, in fact, the gills of many species of Melanosporæ show a tendency to deliquesce. Attention is also called to the fact that while liquefaction of the elements of the hymenium was abandoned

[17] Ann. Bot. 10: 123–184. 1896.

at a very early stage in the evolution of the agarics, it persisted throughout the entire sequence of development in the parallel group of Gastromycetes. Among the Phalloideæ the semi-liquid product has a decided smell and sweet taste, attractive to insects; "thus the feature which proved a failure in the Agaricineæ has been an important factor in raising the Phalloideæ to their present position as head of the fungal subkingdom."—J. M. C.

PROFESSOR T. KIRK has long been a student of the New Zealand flora. Aside from the great interest which attaches to the flora itself, the influence of the presence of man is exceptionally open to study. This phase of the subject was presented recently by Mr. Kirk in a presidential address before the Wellington Philosophical Society, entitled "The displacement of species in New Zealand."[18] Many interesting ecological features of this displacement are presented, some of which deserve mention here. The destruction of the great "kauri" forests has resulted in the absolute desolation of the areas. It seems that the bushmen fired the dead branches after the logs were removed, not only destroying all young growth, but also all fallen seeds, since the soil is charged with resin and becomes intensely heated. Next to the direct operations of man the chief agents in destructive work are sheep and rabbits, whose close feeding has all but extirpated the more delicate plants over large areas. The pig and rat have proved destructive also, and a curious orchid (Gastrodia) is cited whose nutritious tubers are particularly attractive to the black rat, and which has become very rare wherever the black rat is plentiful. A small native beetle has greatly reduced many species of Compositæ by depositing eggs among the disk flowers, the larvæ from which destroy the ovary before it reaches maturity. The great increase of this insect in recent years is thought to be due to the frequent burning of the surface vegetation, thus destroying the lizards and predatory insects which kept the beetle in check. In many cases introduced plants have taken possession of sea-beaches, completely displacing the original vegetation. A most notable case of displacement is that of the New Zealand flax (*Phormium tenax*), *Cyperus ustulatus*, and the common *Pteris esculenta*, all robust plants, by European grasses and clovers. In other cases certain native grasses have succeeded in maintaining themselves associated with the foreigners, "to the great benefit of the stock-grower." Special attention is called to the invasion of three species of Epacris, all natives of New South Wales, which have been observed within the last thirty or forty years to enter New Zealand and rapidly take possession of large areas. So rapidly were they extending their area in the direction of the prevailing winds that Mr. Kirk is convinced that they "would be able practically to replace the indigenous vegetation over the entire area if not interfered with by man.'

[18] Jour. Bot. **34**: 338. 1896.

In this case there is clear evidence of the transportation of seeds by atmospheric currents over a distance of from 1200 to 1400 miles, and of their establishment in a new country. The number of naturalized species has now reached more than five hundred, and if the rate of increase of the last few years be continued for the next fifty years the naturalized and indigenous species will be about equal, a condition of things very unlikely to be reached, as favorable conditions for encroaching species must be reduced rapidly with their increase. The distribution of naturalized plants follows the same lines as that of the indigenous flora, the number rapidly decreasing southward. The Auckland district is much more favorable for the naturalization of plants from warm temperate climates than any other portion of the colony. An interesting illustration of this is given. A large quantity of ballast from Buenos Ayres was discharged at Wellington, and more than one hundred species of plants made their appearance before the close of the second summer, the great majority of which had been naturalized already in the Auckland district. Not more than two, most likely but one, of these will become naturalized on the stiff Wellington clay, while it is certain that fully one-third of them would have become established on the light scoria soil of the Auckland isthmus. Mr. Kirk draws a good distinction between displacement and replacement, and does not anticipate the absolute extermination of any large number of indigenous plants.—J. M. C.

TWO RECENT paleobotanical reports of interest are those upon the flora of the Potomac formation, by Professor L. F. Ward, and upon the Tertiary floras of the Yellowstone National Park, by Mr. F. H. Knowlton. Professor Ward discusses[19] the Potomac formation in general, and then takes up in detail the several floras into which it has been divided. These lower cretaceous floras present some striking features, aside from the ordinary lists of paleobotanical material. Certain specimens are thought to be the remains of a species of Casuarina, which has been called *C. Covillei*. The plate representing a single specimen of this plant also contains the figures of two living species of Casuarina, and the resemblance certainly is striking; but with Ephedra and other jointed and fluted fossil genera in the background, to say nothing of Equisetum and its associates, the certainty of this reference is not convincing. As the author remarks, "it would certainly be an interesting fact if it were proved that this anomalous type of vegetation lived in America during lower cretaceous time." Even if the genus did occur here, however, it is not so clear that its association with Ephedra is at all significant of an intermediate position between gymnosperms and angiosperms, or that angiosperms have been derived from gymnosperms. The most interesting of the ferns are the species of Thyrsopteris, a living genus of but a single

[19] Fifteenth annual report of the U. S. Geol. Survey, 307. 1895.

species and confined to the island of Juan Fernandez. The conifers are abundantly represented, and among them the new genus Nageiopsis, so closely resembling a cycad. Naturally, Sequoia occurs in the formation, as it seems to in cretaceous and tertiary deposits over nearly the whole globe. The dicotyledons are abundant enough, but the monocotyledons are very rare, but seven forms being referred to them doubtfully. Attention is also called to the important part played by the genus Populus in the geological history of plants. It is one of the most widespread genera of fossil plants, and seems to have developed along several distinct lines, and, "historically considered, is the most interesting of all dicotyledonous genera."

Mr. Knowlton's paper[20] is a brief preliminary statement concerning a full report which will appear later. It seems that the most remarkable fossil forest known occurs in the Yellowstone National Park, and it has yielded abundant material in excellent condition for study. The author contrasts the flora of the park today with this tertiary flora. "The dominant elements of the living flora are the abundant coniferous forests, but these involve a very meager display of species; the tertiary forests, however, were characterized by the dicotyledonous trees, such as walnuts, hickories, oaks, beeches, chestnuts, elms, magnolias, sycamores, sumacs, lindens, azalias, persimmons, and ashes. There seems to be little relation between the two floras, and they are certainly not related by descent. The tertiary flora has its affinities at the south, while the present flora is evidently of northern origin."—J. M. C.

[20] Amer. Jour. Sci. IV. **2**: 51. 1896.

NEWS.

DR. N. BUSCH, of Dorpat, is about to make a botanical investigation of certain unexplored regions in northern Caucasus.

DR. F. KOHL, assistant professor of botany in the University of Marburg, has been advanced to the professorship.

DR. K. MÜLLER, privat-docent in the technical high school in Berlin, has been appointed assistant professor of botany.

DR. M. WESTERMAIER, professor of botany in Freising, has been appointed to the same chair in the University of Freiburg, Switzerland.

MR. J. H. MAIDEN has been appointed Government Botanist and Director of the Botanic Gardens at Sydney, to succeed Mr. Charles Moore.

IN THE RECENTLY organized department of biology in the graduate school of Georgetown University Mr. M. B. Waite has been appointed professor of botany.

MR. W. ALPHONSO MURRILL has recently discovered the rare *Asplenium ebenoides* at Blacksburg, Va. His field notes are published in the *Linnæan Fern Bulletin* for October.

DR. A. ZIMMERMANN, privat-docent in the University of Berlin, has gone to the Botanic Garden of Buitenzorg, Java, where he began October first his duties as botanist to the new department of coffee culture.

THE RICHEST COLLECTION of palms in the world is said[1] to be in the Botanic Garden at Buitenzorg, Java. It contains three hundred species that are determined, one hundred probably new and still undescribed, and one hundred varieties of known species.

THE BOTANICAL SOCIETY OF AMERICA, at its recent meeting in Buffalo, took the following action in reference to the death of Mr. M. S. Bebb:

"The Botanical Society of America desires to place upon record an expression of esteem for its deceased member, Michael Schuck Bebb, who died December 5, 1895, at San Bernardino, California. His published studies upon the difficult genus Salix have brought him to high rank as a professional botanist, and American botany owes to him a debt of gratitude as one of its most distinguished representatives."

[1] Garden and Forest 9:360. 1896.

A NEW QUARTERLY devoted to the art of brewing made its appearance in July. It is the *Journal of the Milwaukee Brewing Academy*, edited by Alfred Lasché. It is likely to contain matter of interest to bacteriologists and students of yeasts and other simple fungi. Considerable improvement in typographic style ought to be made, and in particular the authorship of the articles ought to be indicated. The first number of the *Journal* consists of 48 pages. Its price is $5.00 per year.

THE RUST (*Puccinia Asparagi*) which affects the asparagus plant has become so abundant in parts of New Jersey as to cause much alarm among growers of this vegetable. The State Experiment Station has just issued a circular advising the burning of affected plants. It will be interesting to learn how widespread and abundant the rust is at the present time in the United States. Botanists who have observed it will do a favor by reporting to Dr. B. D. Halsted, New Brunswick, N. J., or to the BOTANICAL GAZETTE.

TWELVE STATES have laws of some sort for the prevention of the spread of plant diseases. They are as follows: California, a general law; Connecticut, peach yellows; Delaware, peach yellows; Kentucky, black knot of plum and cherry; Maryland, peach yellows; Michigan, peach yellows and black knot of plum and cherry; New Jersey, for a cranberry disease, and of general application under special conditions; New York, peach yellows and black knot; Oregon, a general law; Pennsylvania, peach yellows; Virginia, peach yellows; Washington, a general law. These various laws are given in full in a compilation prepared by Erwin F. Smith and printed as Bulletin 11 of the Division of Vegetable Physiology and Pathology, Department of Agriculture.

RECENT ANNOUNCEMENTS of new books of botanical interest to be published by The Macmillan Co. in the course of the winter include *An elementary botany for high schools*, by L. H. Bailey, professor of horticulture in Cornell University, with numerous illustrations by Holdsworth; *The survival of the unlike*, by the same author; *Physiology of plants*, by J. C. Arthur, of Purdue University; *Grasses*, by W. H. Brewer, of Yale University; *Bush fruits*, by F. W. Card, of the University of Nebraska; *Plant diseases*, by B. T. Galloway, E. F. Smith and A. F. Woods, of the U. S. Department of Agriculture; *Seeds and seed growing*, by G. H. Hicks, of U. S. Department of Agriculture; *Leguminous plants*, by E. H. Hilgard, University of California. All but the first two are to be issued in the Rural Science Series.

MR. J. G. JACK will conduct a series of fifteen lectures and field meetings at the Arnold Arboretum during the autumn for the purpose of supplying popular instruction about the trees and shrubs which grow in New England. The class will assemble each day in the lecture room of the Bussey Institu-

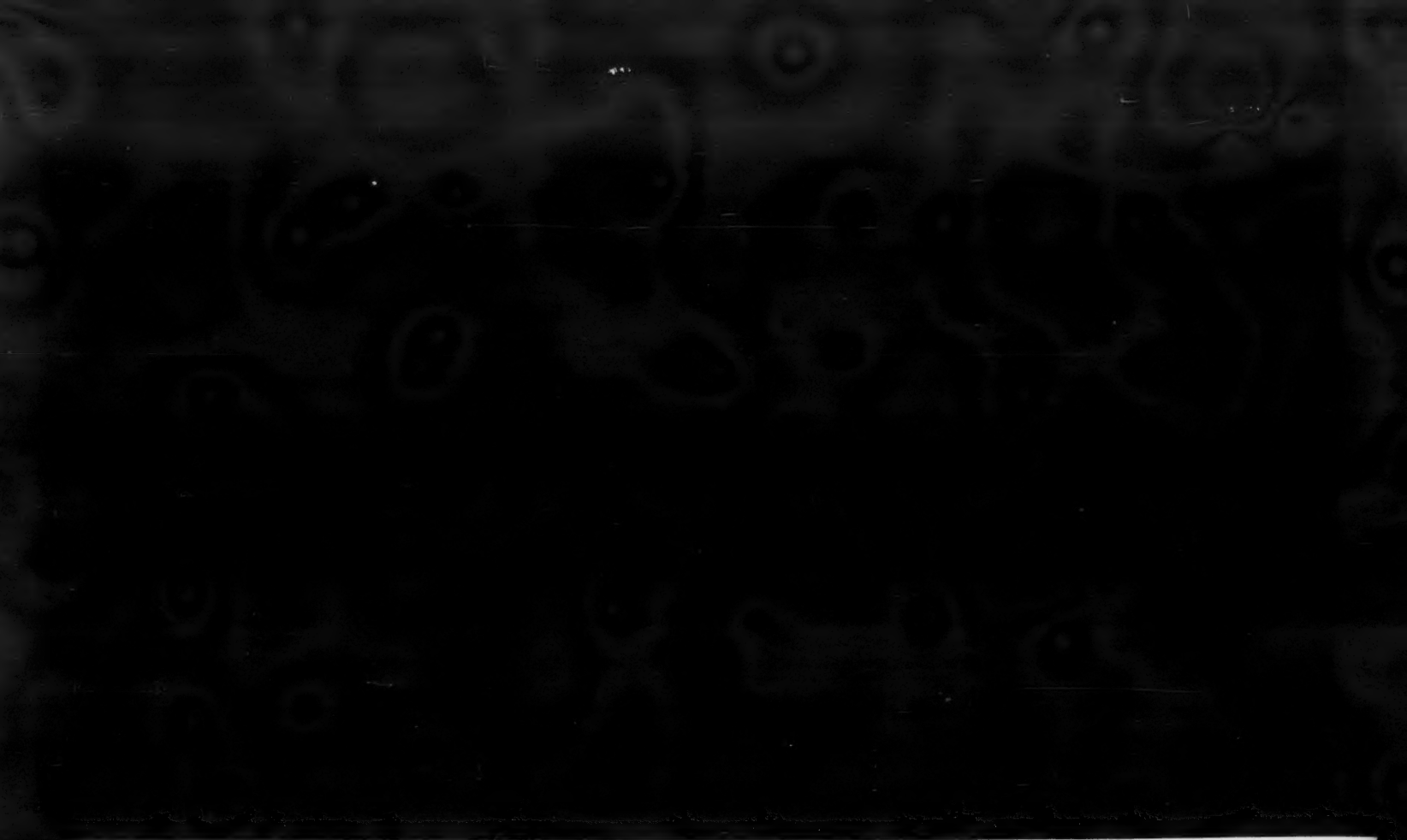
tion, where a review will be given of certain groups of trees and shrubs. It will then adjourn to the Arboretum for an informal outdoor study of the plants. The instruction given in these meetings is not to be technical, but the intention is to indicate by comparison the easiest means of distinguishing the common native trees and shrubs as they appear in eastern Massachusetts, and of recognizing the foreign species which have been introduced into our gardens. An hour and a half to two hours will be devoted to each meeting. During the season the class will meet once or twice outside of the Arboretum at some favorable place for the study of trees.

THE FOLLOWING report was presented at Buffalo to the Botanical Club by the committee on nomenclature, and adopted:

The committee on nomenclature, which was requested at the Springfield meeting to prepare a report, would respectfully submit the following preamble and resolutions:

WHEREAS, A large number of requests for a list of all North American Pteridophyta and Spermatophyta has been received, and publication of such a list, when prepared, has been informally offered by the Assistant Secretary of the Smithsonian Institution,

Resolved, That the committee be and hereby is authorized to prepare for publication a list of Pteridophyta and Spermatophyta occurring in the United States and the British Possessions of North America.

Resolved, That the committee be and hereby is authorized to prepare and publish a supplement to the "List of Pteridophyta and Spermatophyta of northeastern North America," such supplement to contain additions and published corrections to the list. Such publication has been promised by the Editor of the Torrey Botanical Club.

Resolved, That the committee be and hereby is authorized to prepare a fuller statement of the rules adopted at the Rochester and Madison meetings, with examples illustrating their operation, and submit it to the club at a subsequent meeting, for publication in the proposed List of North American Pteridophyta and Spermatophyta.